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Radiation Bio-Effects

Summary Report

January-December 1970

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Public Health Service

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PHS 1809	Radiation Bio-Effects Summary Report, January - December 1967 (PB 183 796)
PHS 1809	Radiation Bio-Effects Summary Report, January - December 1968 (PB 183 797)
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DBE 69-2	Evaluation of a Possible Causal Relationship between Fallout Deposition of Strontium 90 and Infant and Fetal Mortality Trends (October 1969) (PB 188 974)
BRH/DBE 70-1	Radiation Bio-Effects Summary Report, January - December 1969 (PB 190 110)
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This report summarizes the activities of the Division of Biological Effects during 1970. The investigations of theEpidemiologic StudiesBranch and the Experimental Studies Branch which comprise the Divisionare disucssed. Titles of papers that division investigators have prepared for presentation and those appearing in print during the year are listed. (BRH abstract)

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Summary Report
January - December 1970

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Division of Biological Effects

Compiled and Edited by
Donald M. Hodge

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of Radiological Health
Rockville, Maryland 20852

NOTICES

This report contains preliminary data and was prepared primarily to record the year's work, and is not intended as a substitute for publication in the open literature. It is subject to revision and does not represent a final report. Please do not quote from or refer to this work in open literature without permission from individual authors to reference the material as a personal communication.

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FOREWORD

The Bureau of Radiological Health conducts a national program to limit man's exposure to ionizing and nonionizing radiations. To this end, the Bureau (1) develops criteria and recommends standards for safe limits of radiation exposure, (2) develops methods and techniques for controlling radiation exposure, (3) plans and conducts research to determine health effects of radiation exposure, (4) provides technical assistance to agencies having radiological health programs, and (5) conducts an electronic product radiation control program to protect the public health and safety.

The Bureau publishes its findings monthly in *Radiological Health Data and Reports*, appropriate scientific journals, and from time to time in Division-sponsored technical reports.

The technical reports published by the Division of Biological Effects contain information generated by the Division staff which is timely and useful to the radiological health program. Subjects covered by these reports are varied, for the Division is charged with developing--through animal investigations and population studies--knowledge of the biological effects of radiation delivered to man from his environment and from his use of substances and devices that emit radiation. The reports are distributed to persons and repositories that have expressed an interest in the biological effects of radiation; in addition, the reports are available from the National Technical Information Service.

Readers are encouraged to report omissions or errors to the Bureau. Additional comments or requests for further information are also solicited.

John C Villforth

John C. Villforth
Director
Bureau of Radiological Health

PREFACE

Through human epidemiologic studies and experimental animal investigations, the Division of Biological Effects conducts an active research program aimed at examining the biological effects of radiation on human health. Radiations of the entire electromagnetic spectrum are under the purview of the division, as are certain particulate emissions and vibrations emitted by sonic, infra- and ultrasonic devices. Of particular interest are low-dose effects that might be experienced by large segments of the population.

This report summarizes the research findings of the division during 1970. It is intended to familiarize the reader with the division's activities and to place in the public domain the most recent findings in an important health area.

The research program would have been unable to develop along its present lines without the guidance of its advisory committees. It is with gratitude that I wish to acknowledge the contributions of these committees whose members are listed in Appendix C. I should also like to express my deep appreciation to the entire staff of the division (Appendix D) for their time and devotion in building the division during the past several years.

A new agency, the Environmental Protection Agency, has recently been established. The Division of Biological Effects has been split to enable both the EPA and DHEW to have a competency in radiation biology. Subsequent reports will reflect this change.

William A. Mills

William A. Mills, Ph.D.
Director, Division of Biological Effects
Bureau of Radiological Health

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DIVISION OF BIOLOGICAL EFFECTS

Dr. William A. Mills, Director

During 1970 the Division of Biological Effects, Bureau of Radiological Health, has continued its biomedical research program to examine the association between various aspects of radiation exposure and public health. The division investigates the biological effects of ionizing and nonionizing radiations present in the environment, and emanating from consumer products, as well as the effects of ultrasonic vibrations as they relate to the health and well-being of man.

The division is organized into functional sections as depicted in Figure 1. The organizational lines are not rigid, allowing a great deal of interchange between sections. Nearly all of the studies in the Experimental Studies Branch employ animal species, and conversely, nearly all of those in the Epidemiologic Studies Branch examine human populations. Both branches strive to relate the meaningful effects of radiation exposure to human health.

Most of the division's investigations are conducted at its Rockville, Maryland, laboratory. However, a large long-term study, carried out by contract, was initiated several years ago at Colorado State University, Fort Collins, and is slated to continue its productive investigations on ionizing radiation in the young animal as it relates to human exposure. Another aspect of this problem, but employing internal emitters, is examined under contract at Falls Church, Virginia, some 20 miles from the Rockville facility. Various other contract studies are in progress with universities, hospitals, and other public and private institutions. A few studies extend to other government laboratories by special agreements. The division also employs the foreign currency program of Public Law 83-480 to secure information from uniquely qualified biomedical researchers in foreign countries.

The studies summarized in this annual report in many cases give preliminary results that have not been published; in other cases they reiterate published findings. Papers published by division authors and papers submitted for oral presentation during the year are given in Appendixes A and B.

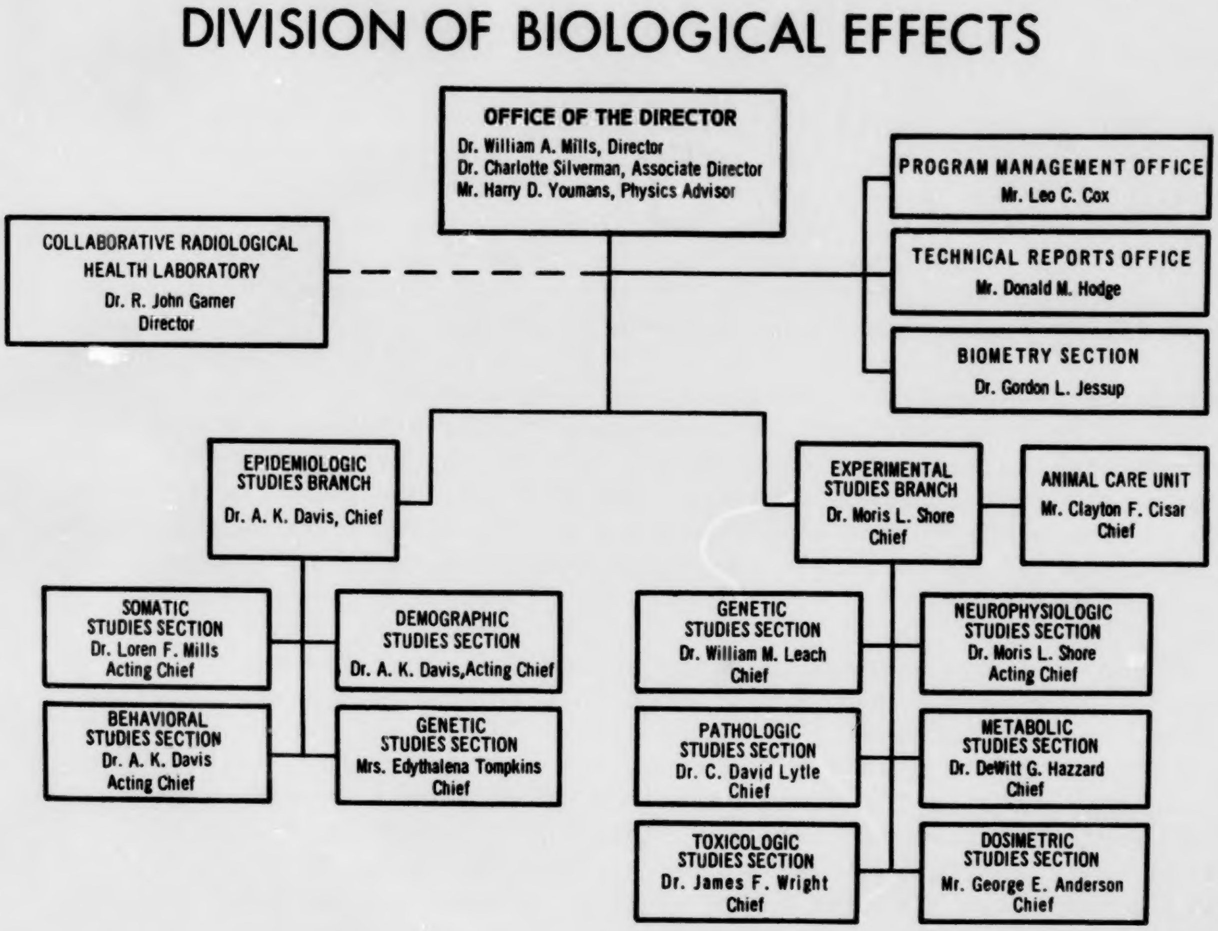

Figure 1. Organization of the Division of Biological Effects.

BIOMETRY SECTION

Dr. Gordon L. Jessup, Jr., Chief

The Biometry Section provides statistical and mathematical consultation, experimental design, and data analysis services to all sections of the division for a variety of radiobiological investigations. Statistical design and analysis of experimental data are essential if generalized conclusions are to be drawn and inferences made about populations from small sample results. The section also develops mathematical models to characterize physical or biological systems, and cooperates with the investigator to define experimental objectives and variables, formulate mathematical functions, define and estimate the components of variance, and synthesize number systems to characterize, compare, and evaluate individual factors and their interactions. Results of these services are reported to the investigators in a biometric analysis which describes the analytical methods used and the conclusions that can be drawn.

A major effort of the Biometry Section in 1970 was devoted to a review of the human and animal radium toxicity literature. This review was undertaken to evaluate the present state of knowledge and to determine to what extent the presently available information is capable of resolving the questions concerning linearity of the dose-effect relationship and the existence of a threshold for radiation effects. The conclusions resulting from this review were:

(1) It is important to distinguish between dose-response models which attempt to describe radiation effects as accurately as available information permits, and public health protection standards which, recognizing that the real health effects are incompletely known, make allowance for margins of safety that are considered to be reasonable and prudent.

(2) There do not appear to be sufficient data to specify an unequivocal dose-response model or to establish whether or not there is a threshold for radium injury at low levels of body burden.

On the basis of these conclusions, it was recommended that public health authorities should continue to follow the philosophy adopted by the International Commission on Radiological Protection, National Council on Radiation Protection and Measurements, and Federal Radiation Council of a linear, non-threshold model for recommending acceptable limits of human radiation exposure to radium. This recommendation does not imply that proof of a linear, non-threshold model has been demonstrated, nor does it constitute rejection of the possible existence of a threshold. It is merely the most cautious and reasonable approach, in the absence of proof to the contrary, to assume that any radiation exposure has an accompanying biological risk.

COLLABORATIVE RADIOLOGICAL HEALTH LABORATORY[1]

Dr. R. John Garner, Director

Introduction

The Collaborative Radiological Health Laboratory was conceived in 1962 as a joint operation between the sponsoring agency, the Division of Biological Effects of the Bureau of Radiological Health, U. S. Public Health Service, and the Department of Radiology and Radiation Biology, Colorado State University. Its primary purpose is to conduct a long-term study of the effects in the beagle of a single exposure to low levels of gamma radiation with emphasis on investigation of the radiosensitivity of the unborn and young animal in terms of long-term effects. For this purpose, an experiment, ultimately to involve approximately 1,700 dogs, was initiated in December 1967. It had always been recognized that supporting studies would be necessary, for example, to permit development of techniques for use in the principal experiment, to provide supplementary information on the more immediate effects of irradiation at the ages of interest in this experiment and, not least, to fulfill the needs of investigators for publishable material. These supporting studies have been designed, not only to provide information of direct relevance to the "Long-Term Experiment" but also to ensure the most economical use of animals.

Microwave studies, introduced in 1969, have continued during 1970. During this year, a small experiment was mounted on the teratogenicity of cyclohexylamine, a metabolite of cyclamate. This marks the introduction to this laboratory of research on a noxious agent other than radiation.

Major Programs and their Interrelationships

Four major programs are currently under way. The Long-Term Experiment, the major research project, is designed to examine the long-term effects in the beagle of a single whole-body exposure (0 R, 20 R, or 100 R ^{60}Co gamma radiation) delivered over a 10-minute period at 8, 28, or 55 days postcoitus or 2 days, 10 weeks, or 1 year postpartum. As of October 31, 1970, a total of 975 animals had been assigned and it is now estimated that commitment, originally planned to take 5-6 years, will be completed by September 1972.

The Pilot Age-Sensitivity Study was mounted to provide, on the one hand, information on the acute effects of irradiation at some of the ages of interest in the Long-Term Experiment and, on the other, a preview of events which can be expected to occur in the latter experiment. The circumstances of irradiation, and ages at exposure (confined to 8, 28, or 55 days postcoitus or 2 days after birth) are identical to those of the Long-Term Experiment but exposures are in the LD_{50} range. Animals surviving the first 60 days of postnatal life are scheduled to be kept for up to 4 years, again under management procedures identical to the Long-Term Experiment. This study can thus be regarded as an extension of the latter experiment and allows dose-response estimates to be made over a wider range of exposures. Of the minimum of 300 dogs planned to be assigned to the second part of the experiment, as of October 31, 1970, 251 had been committed. Fifty-nine animals scheduled for sacrifice at 70 days had been necropsied and 2 at 2 years.

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[1] A detailed account of the work at the Collaborative Radiological Health Laboratory is given in the Annual Report for 1970, Colorado State University - U.S. Public Health Service Collaborative Radiological Health Laboratory, Fort Collins, Colorado, (Contract PH 86-62-135).

Animals produced for, but for various reasons not assigned to, the Long-Term Experiment are utilized in a Recovery Study designed to assess recovery in the 10 weeks following pre- or neonatal exposure to 100 R. The extent of "remaining injury" at 72 days in animals receiving 100 R at 2 days is tentatively assessed at 23 percent.

As commitment to the Long-Term Experiment nears completion it is becoming possible to reduce the size of the breeding pools set up to provide a constant flow of pups for this experiment. A program is under way to eliminate all F_2 dogs from the colony and, to achieve maximum utilization, F_2 bitches are assigned to terminal studies as they come into estrus. These animals, and a smaller number of males removed with them, have contributed information to a variety of studies, many of which are included in the subsequent sections.

Growth and Development

The effects, both early and late, of exposure of the beagle conceptus to ionizing radiation occupy a position of primary importance in this laboratory. For a better understanding of these effects it has been necessary to define more clearly normal development in the beagle. During the past year events occurring prior to implantation have been examined. Ovulation occurs approximately 1 day after first acceptance. Tubal passage requires 8-12 days, and during this time development proceeds from the primary oocyte to the morula stage. The embryos spend approximately one additional week in the free-floating blastocyst stage. Implantation, first indicated by local endometrial edema, occurs, on average, 17-18 days after breeding. Three methods for determining prenatal age have been evaluated. Timing based on changes in vaginal cytology has been found to provide a much better correlation with development than either timing from breeding or refusal to breed.

By combining data from the Long-Term and Pilot Age-Sensitivity Studies with those from supplementary experiments it has been possible to assess the effects of prenatal irradiation over a wide range of exposures. A consistent and marked dose-dependent reduction in litter size at 14 days was noted for irradiation at either 8 or 28 days postcoitus (dpc) with exposures from 20-270 R. A similar response occurred at somewhat higher exposures for animals irradiated at 55 dpc. A dramatic increase in the incidence of major developmental malformations in dogs irradiated at

28 dpc was observed with exposures as low as 150 R. No gross malforming effects were apparent following irradiation at either 8 or 55 dpc.

Parameters of growth include head dimensions and growth of the lumbar spine. A preliminary analysis of data from the Long-Term Experiment indicates that there may be a reduction in skull length in animals receiving 100 R prenatally.

It is estimated from lumbar spine data (obtained from animals in the Long-Term and Pilot Age-Sensitivity Studies and, so far, subjected only to partial analysis) that exposure at 28 dpc may result in a reduction in the length of the total lumbar spine at 90 days of 0.1 percent/R and, at 2 days postpartum, of 0.01 percent/R. These results, and those reported above, provide strong evidence that effects may be demonstrable at the exposure levels (20 and 100 R) used in the Long-Term Experiment.

In anticipation of further work on irradiation effects on bone, the value of physical parameters such as volume, specific gravity, and ash content for following bone changes is under assessment.

The knowledge acquired in this laboratory over the past years on prenatal development in the beagle led to a pilot investigation following an expression of interest by the Food and Drug Administration, of the teratogenicity of cyclohexylamine, a metabolite of cyclamate. When compared with untreated controls, bitches receiving 32 mg/kg body weight of cyclohexylamine hydrochloride per os daily from the sixteenth through the thirty-first day of gestation gained slightly less weight during pregnancy and produced approximately the same number of fetuses which, however, weighed less and were slightly shorter than control pups. No significant increase in the number of developmental malformations resulted from the treatment. It was concluded that under the conditions of this experiment cyclohexylamine was non-teratogenic in dogs but may have resulted in slight in utero growth retardation.

Studies on serine dehydrase have continued. This enzyme in liver seemed to offer a suitable system for examining the delayed effects of prenatal irradiation upon enzyme ontogenesis and postnatal induction. However, considerable variations occur in the rate of development of the enzyme in individual Sprague-Dawley rats after birth and, although contributory factors have been indentified and controlled, these variations remain great enough to preclude acquisition of definitive data.

Variations in starvation induction of serine dehydrase have now been observed. The ability of the enzyme to respond to starvation appears to be lost at or before about 12 weeks of age in the Sprague-Dawley strain. The intention had been to extend these pilot studies in rats to the beagle, but preliminary indications are that there is little, if any, serine dehydrase present in beagle liver.

The Long-Term Experiment is intended to yield information on the relative radiosensitivity of the beagle at various stages of development in terms of late effects such as life-shortening. Information on postnatal changes in age sensitivity in terms of acute lethality is being derived from a number of ongoing studies which involve LD_{50} determinations. Current estimates of $LD_{50/60}$ (mid-line in-air exposures) are: 2 d., 385 R; 15 d., 370 R; 35 d., 300 R; 63 d., 277 R; 70 d., 288 R; 1 y., 316 R; 4.5 y., $<$ 280 R. These data provide additional evidence that there are quite definite species differences in age-related radiosensitivity.

Nervous System and Special Senses

CNS-related research in this laboratory is primarily directed towards replacement of the, usually indirect and time-consuming, techniques of the experimental psychologist by direct monitoring of the brain. Some interesting developments in this area were made during 1970. Data from EEG's recorded from six electrode sites in dogs ranging in age from 5 days to 6 months have been cross correlated using a digital computer. Quantitative changes in both the cross-correlation function and its transform, the cross-energy spectrum, were clearly evident. This provides an objective and quantitative means of testing the level of brain development in the beagle.

More conventional techniques have been applied in microwave studies. Exposure of Sprague-Dawley rats to 2.45 GHz microwave radiation immediately prior to running them in a Y-maze significantly improved their performance. It is suggested that irradiation may produce effects similar to a variety of CNS stimulants which act by facilitating consolidation of the memory trace.

Beagle pups, 10 weeks old, tested in an approach runway after whole-body exposure to 2.45 GHz microwaves, showed no appreciable changes in performance and/or motivation.

Electroencephalograms have been recorded from approximately 150 dogs in the Pilot Age-Sensitivity Study. These are currently undergoing analysis using auto- and cross-correlation techniques to assess any changes in rhythmicity and site synchronization. Simultaneously sensory and autonomic function is being studied by means of visual and auditory evolved responses and cardiac responses to light and sound (see below).

An electrophysiological method for determining auditory acuity was developed during 1969. In order to check the reliability of this method it was decided also to employ a more conventional approach, shock avoidance conditioning to tone stimuli. Method development has now been completed.

The possibility of using changes in heart interval in response to light and tone stimuli as a simple method of evaluating vision, hearing, and autonomic tone is being evaluated.

This study led to development of a rapid and readily applied method for determining heart rate. A survey of heart intervals in dogs irradiated perinatally revealed a difference in interval from that of control dogs at 90-180 days of age. The heart interval in females was also found to be significantly shorter than in males.

All animals in the Long-Term Experiment periodically undergo clinical eye examinations. Only minor lesions have been observed, the significance of most of which is doubtful. It is possible that the presence of early lens opacities involving the suture lines may prove to be an exception. No ocular lesions were observed in a small number of pups which had received microwave irradiation 7 days previously.

Urinary System

The kidney is expected to be an organ of prime interest in the context of late radiation effects and, accordingly, the discovery in 1969 of focal interstitial nephritis, pyelitis, or pyelonephritis in colony puppies caused some concern. A survey indicates that the majority, if not all, pups aged 19-22 weeks are so affected. The cause of the inflammation is not known. It is first seen at 13-14 weeks, is most severe at 19-21 weeks and begins to undergo resolution at about 22 weeks. The possibility that the inflammation is related to treatment received by all colony animals is being investigated.

A survey of F_1 and F_2 generation animals (now of average age about 7 and 5-1/2 years respectively) has revealed that 59% of these, the oldest dogs in the colony, had urine protein levels of 1+ or greater. The cause of the proteinuria is unknown but intercapillary glomerulosclerosis was a universal finding postmortem. Heavy loss of protein tended to be associated with more severe pathologic changes suggestive of a mild glomerulonephritis. No dog had clinical signs of renal disease other than proteinuria.

In an attempt to correlate morphologic and functional changes occurring serially, a renal biopsy technique has been developed and this is now being used in conjunction with a simple phenolsulfonphthalein test of renal function.

The possibility of using various proposed indices of tubular function which do not require complete collection of urine is also being explored. A comparison of inulin and endogenous allantoin clearances suggests that allantoin may be suitable as a reference material for this purpose since it is apparently not elaborated by the tubules.

Evidence is presented from two studies involving exposure of late fetal or early postnatal dogs to sublethal whole-body irradiation which indicates that damage to the kidneys may be sufficiently severe in some animals to produce terminal uremia within the first year of life. The alterations appeared to involve the glomerulus primarily, particularly the mesangial region. Tissues collected at scheduled times from clinically healthy dogs similarly exposed have revealed varying amounts of glomerular damage which was generally proportional to the exposure received and the time elapsed after irradiation.

Ongoing studies of radiation effects following perinatal exposure are being complemented by ultrastructure studies on postnatal nephrogenesis in the beagle.

A practical problem in work on the urinary system is acquisition of a urine sample from a dog "on demand." An electrical stimulation method was developed during 1969 and has now been put into routine use with excellent results.

Endocrine System

Of the several organs of the endocrine system, to date only the thyroid gland has been found to be the site of significant disease in CRHL beagles. Various tests of thyroid function have been applied. The ^{125}I-T4 test appears to be unreliable in the dog; the ^{125}I-T3 test has shown better correlation with physical state. Serum cholesterol values above 390 mg percent were also indicative of hypothyroidism but about half the animals in which this condition was diagnosed clinically showed values of less than 280 mg percent.

An investigation has begun into the etiology and pathogenesis of the spontaneous thyroiditis observed in the colony. Attempts are also being made to reproduce the condition by inoculation of thyroglobulin extracts into normal dogs.

Another approach to the detection of hypothyroidism is to make use of peripheral manifestations such as changes in EKG and EEG which have been recorded in man but apparently not investigated in the dog. As a preliminary, EKG, EEG, auditory and visual evoked potentials, aortic pulse-wave velocity and blood pressure have been recorded from five animals with physical and laboratory evidence of hypothyroidism. These have not yet been analyzed.

An investigation of the effect of microwaves on testicular function in the beagle is planned which requires exposures under pentobarbital sodium anesthesia. It has been shown that anesthesia significantly reduces plasma testosterone levels over a period of 3 hours; corticosteriod levels are not affected.

The Beagle Colony: Clinical and Pathological Observations

The cesarian-derived, controlled-access beagle colony, which is the primary source of experimental material for this laboratory, comprised, as of October 31, 1970, 2,118 animals. Of these about 1,900 were animals assigned to one or other of the four major programs previously summarized; 45 were F_1 animals retained as a pilot aging population and the remainder were either puppies less than 14 days old awaiting assignment to the Long-Term Experiment or animals assigned to subsidiary studies. Some pertinent statistics are: Conception rate, 87 percent; average litter size at birth, 5.5; mortality through day ten, 20.4 percent.

The disease status of the colony continued to be monitored through 1970 by postmortem examination of dogs dying spontaneously or killed because of serious illness. The colony suffered an outbreak of canine distemper during this period which was rapidly controlled. Polyarteritis nodosa was observed for the first time. Six older bitches developed squamous cell carcinomas of the nonpigmented skin of the posterior abdomen. Additional cases of cor pulmonale, glomerulonephritis, hemolytic anemia and hypothyroidism were observed.

Behavioral changes and histologic features of the genital system have been examined during various stages of the bitch's estrus cycle in an attempt to relate these features to the hormone levels which control the cycle. On the basis of these observations, the canine estrous cycle has been redefined in terms applied to other species. This system allows the comparative aspects of the canine estrous cycle to be more easily understood.

The distribution of ages for onset of first estrus in bitches in the colony has been re-evaluated. The new estimate of the mean time to first estrus is 295 days, with 0.95 confidence limits of 291-299 days.

An inverse correlation between the length of the gestation period and litter size has been demonstrated. Litter order also appears to affect the gestation period.

Sperm counts are routinely performed in connection with the various breeding programs. The feasibility of using the Coulter Counter to replace the conventional hemacytometer technique has been demonstrated.

EPIDEMIOLOGIC STUDIES BRANCH

Dr. A. K. Davis, Chief

The Epidemiologic Studies Branch attempts to define the biological effects of radiation exposure in human populations. The studies include a variety of populations such as occupational groups, large populations exposed to gamma or radionuclides in fallout, and armed service groups exposed to radar or ultrahigh frequency broadcast devices. Medical or hospital patients exposed to x or gamma radiation for diagnostic or therapeutic purposes are the major populations under study. The use of radionuclides in diagnosis and treatment of certain diseases also provides populations of interest. Population size may be as small as that of a disability retirement group or as large as the survivors of the nuclear explosions at Hiroshima or Nagasaki.

These populations are chosen because of certain characteristics. For example, different age groups are needed to study the radiosensitivity of the fetus or the thyroid of the growing child; the offspring of irradiated pregnant mothers and the progeny of irradiated fathers are populations of interest; hospital and dental patients as well as certain groups of physicians are all subjects of specific studies performed during this past year.

The methods used in these investigations include morbidity and mortality statistics from hospital and armed service records, special surveys by questionnaire or interview of population samples, histories and medical examinations of specific members of a population, and pathological examinations of tissue. There are prospective studies in which medical histories are obtained and the course of disease is followed through clinical examinations. Other retrospective studies identify populations having a specific disease and the characteristics of the population are examined through medical records or health statistics.

Various means are used to achieve the goals of these studies. The in-house statistical and medical staff of the branch are the primary strength of the program. The in-house effort is supplemented through contracts or research grants with state health departments, medical institutions, or other public or private organizations.

Until recently, ionizing radiation has been the only concern of the branch. However, in the last year, projects to study nonionizing radiation, particularly microwaves, have been initiated. This brief experience indicates a broad ignorance of possible biological effects, and a major effort will be required to detail the biological effects of nonionizing radiations in human populations.

GENETIC STUDIES SECTION
Mrs. Edythalena Tompkins, Chief

The Genetic Studies Section examines populations known to have been exposed to ionizing radiation that may have induced some long-term genetic and somatic effects. Cooperation has continued with the Atomic Bomb Casualty Commission in the studies of survivors of the Hiroshima and Nagasaki bombings. Preliminary reports of no increase in leukemia following exposure to therapeutic levels of iodine 131 were confirmed by analyses of data for the total study population. Studies of children exposed to fresh fallout from the Nevada Test Site continue to show no significant increase in thyroid abnormalities. A study of children exposed to iodine 131 for medical diagnostic tests has been planned to provide more definitive answers on the long-term effects on the young human thyroid of exposure to iodine 131. Preliminary data suggest that exposure of the ovaries to therapeutic x ray increases the number of skeletal and/or central nervous system anomalies in children conceived after the irradiation.

A. COOPERATIVE THYROTOXICOSIS THERAPY FOLLOW-UP STUDY
E. Tompkins

The Cooperative Thyrotoxicosis Therapy Follow-Up Study was initiated in 1961 to investigate the incidence of leukemia in patients who had been treated with iodine 131 for hyperthyroidism. In the past, several studies had demonstrated an association between acute exposure to external radiation and an increased incidence of leukemia. However, no data existed on the incidence of leukemia following exposure to relatively low dose - low dose rate radiation. The study design was based on the ability to detect a difference between the leukemia incidence rate in patients treated with iodine 131 for hyperthyroidism as compared to that observed in patients treated surgically for the same disease during the years 1946 through 1964. Abstracts of the medical records of every patient treated for hyperthyroidism during the study period in 25 medical institutions in the United States and one in England provided the base data on the ultimate population of 36,043 persons. These abstracts gave data on the course of hyperthyroidism and its treatment, all known exposure to radiation and a general disease history. All but 437 patients of the study population (1.2 percent) were located; information to update the histories including data on additional treatment for hyperthyroidism outside of the participating centers, interim radiation exposure, and disease history was obtained. When it was learned that a patient had died, a death certificate was obtained. Data collection ceased in mid-1968 and analysis of the observed leukemia rates demonstrated no association between an increased risk of leukemia and treatment for hyperthyroidism with iodine 131.

Although the size of the population to be studied was based on the projected rates of leukemia to be observed, the study design (a population-based prospective study) permitted the analysis of the data for other disease states which might be associated with exposure to low-level radiation as well as the testing of hypotheses about the disease of hyperthyroidism and its treatment. Current analyses are investigating the possible association of exposure to therapeutic doses of iodine 131 and the subsequent development of malignant neoplasms of the lymphatic and hematopoietic tissues (excluding leukemia), thyroid neoplasms, other malignancies, and permanent hypothyroidism. In most analyses the patients are classified according to the treatment which they received for hyperthyroidism. The four treatment groups with the number of patients and the patient years of observation are shown in Table 1. The age-sex distributions are not the same in all treatment groups nor is the average length of followup, so the standard life-table technique with age and sex adjustment has been the primary analytical tool.

TABLE 1. COMPOSITION OF THE STUDY POPULATION

Treatment for Hyperthyroidism	No. of Patients	No. of Patient Years
^{131}I (No thyroidectomy)	19,493	152,000
Surgical Thyroidectomy (No ^{131}I)	11,204	142,000
^{131}I and Thyroidectomy	3,557	32,500
Goitrogens and/or Iodides only	1,359	12,000
Total	35,613	338,500

Results

Incidence of Malignant Neoplasms of the Hematopoietic Tissues

There were 47 patients who developed leukemia following iodine-131 treatment for hyperthyroidism. An additional 38 patients developed other malignancies of the hematopoietic tissues after their treatment for hyperthyroidism. The distribution of these cases by disease, treatment group, and the associated age-sex adjusted rates per 100,000 person-years are shown in Table 2. There is no evidence in these observations to support a hypothesis of an increased rate of neoplasms of the hematopoietic tissues following treatment of hyperthyroidism with iodine 131. Further analyses by sex, type of leukemia, type of lymphoma, and time of the development of the disease post-treatment are also negative. The similarity of the observed rates in the two types of malignancies tend to negate the possibility of a sampling artifact explaining these findings. A manuscript including all of the analyses of the incidence of malignant neoplasms of the hematopoietic tissue is in preparation.

TABLE 2. OBSERVED RATES OF MALIGNANT NEOPLASMS OF THE HEMATOPOIETIC TISSUES

Treatment	No. of Cases	Age-Sex Adjusted Rates/100,000 P.Y.[a]
	Leukemia	
^{131}I	18	8.1 ± 2.0
Thyroidectomy	17	11.0 ± 2.7
^{131}I and Thyroidectomy	10	31.7 ± 11.1
Drugs only	2	-----
	Other Hematopoietic Malignancies	
^{131}I	16	8.2 ± 2.1
Thyroidectomy	18	11.1 ± 2.7
^{131}I and Thyroidectomy	3	7.6 ± 4.4
Drugs only	1	-----

a. Rates ± standard error of rate.

Incidence of Thyroid Neoplasms

Almost 1,500 patients in the study population had a history of surgical exploration of the thyroid 6 months or more after their initial treatment for hyperthyroidism in a study center. The reason for the exploration varied but usually was done because of continued concern about a palpable lesion which failed to disappear after treatment or because of the development of a new lesion. In this group of explored patients only 30 malignant and 98 benign neoplasms were identified pathologically. The treatment of choice for hyperthyroidism for any individual patient is dependent upon many characteristics, but important variables in this choice are the clinical suspicion of the presence of a neoplasm in the thyroid gland and the past history of treatment the patient has received. For these reasons no satisfactory comparison groups can be defined in the study which would permit statistical testing for an increase in one treatment group over another. One can only describe in detail the observations which have been made and carefully define the limitations which must be put on interpretation of the observations. However, the relatively few thyroid neoplasms which have been identified in this large population of patients with known thyroid diseases do lead to the conclusion that if any treatment for hyperthyroidism increases the risk of development of a thyroid neoplasm, such an increase must be minimal. The manuscript describing all of the observations of thyroid neoplasms will be submitted for publication early in 1971.

Incidence of Hypothyroidism

The increasing incidence of hypothyroidism many years after patients were treated with iodine 131 for hyperthyroidism has been a continuing concern of physicians using this mode of therapy. Included in the study population were approximately 6,000 patients who had had no therapy for hyperthyroidism before they were treated with iodine 131 in one of the study centers. The various patterns of dose administered to a patient in the different centers made possible the evaluation of the probability of becoming permanently hypothyroid following a single dose of iodine 131 according to the dose administered to the thyroid gland. The results of this analysis and a similar analysis of patients who were treated by surgical thyroidectomy are shown in Figure 1. These analyses confirm two previously suspected but not documented phenomena: (1) The disease of hyperthyroidism itself has a long-term effect on the function of the thyroid gland as demonstrated in the increasing probability of hypothyroidism with time in the surgical population, and (2) the long-term effect of iodine 131 on the function of the thyroid gland is very dose dependent with the primary difference in the probability of becoming hypothyroid being a function of the number of patients who become permanently hypothyroid in the initial few months post-treatment.

Similar analyses were done to determine the probability of requiring additional treatment to control the hyperthyroidism after a given dose of iodine 131 delivered to the thyroid gland. A comparison of the probability of becoming hypothyroid with that of needing additional treatment for a given dose is then possible and the results at 10 years post-treatment are shown in Table 3.

These data indicate that the optimum dose to be delivered to the thyroid gland to minimize both hypothyroidism and the need for additional treatment is between 76 and 100 μCi. These findings were presented at the International Thyroid Conference in Vienna, Austria, by Dr. David Becker in June 1970.

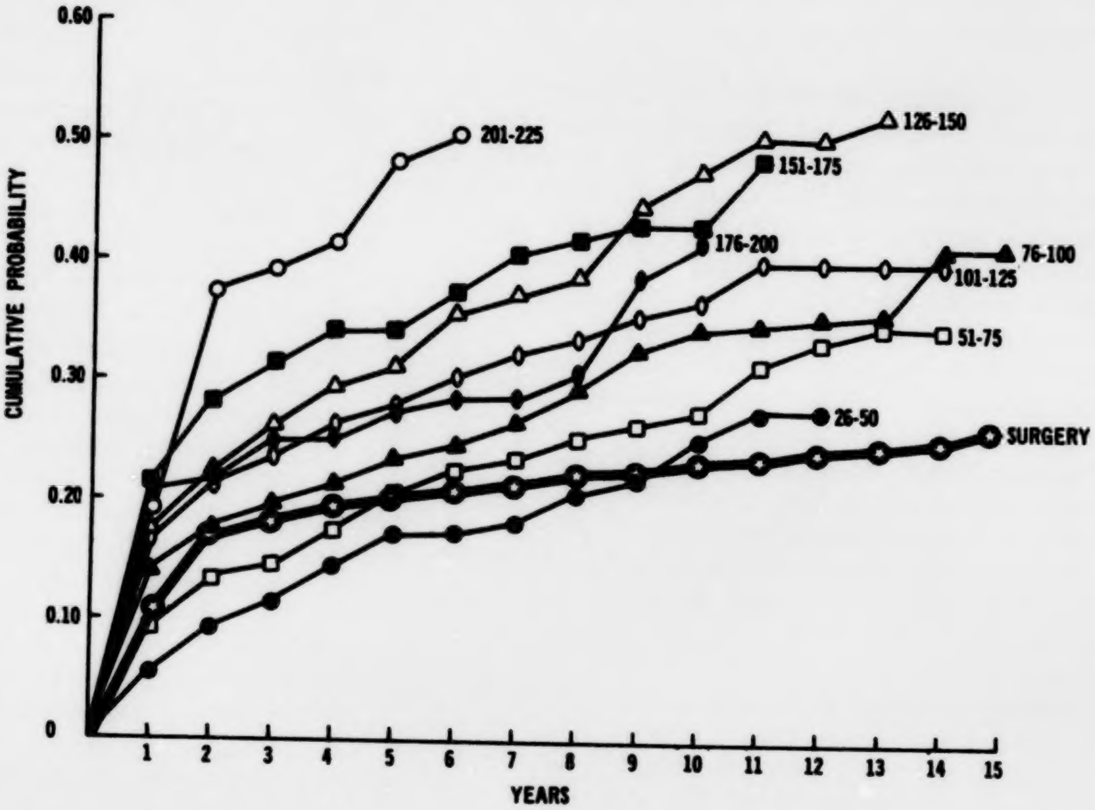

Figure 1. Probability of Becoming Hypothyroid with a Single Treatment (Iodine 131 in μCi per gram).

TABLE 3. THE PROBABILITY OF BEING HYPOTHYROID OR HAVING HAD ADDITIONAL TREATMENT BY 10 YEARS FOLLOWING A GIVEN DOSE OF ^{131}I

Dose Delivered to the Thyroid Gland, μCi	Probability of Being Hypothyroid	Probability of Having Had Additional Treatment
26-50	.26	.61
51-75	.28	.48
76-100	.34	.40
101-125	.37	.42
126-150	.48	.40
151-175	.43	.37
176-200	.42	.41

B. STUDIES OF FALLOUT EFFECTS IN UTAH, ARIZONA, AND NEVADA

Daniel A. Hoffman

The association of x irradiation to the young thyroid and the subsequent development of thyroid neoplasms has been established by various studies. Atmospheric testing of nuclear weapons at the Nevada Test Site produced fallout containing radionuclides of iodine. Since iodine concentrates in the thyroid gland and because several inhabited off-site areas were known to have received "heavy" concentrations of fallout, concern arose as to the possibility that children in these areas received high enough exposure to cause thyroid neoplasms. As a consequence of this concern, the Bureau of Radiological Health has supported studies of the possible effects of radioactive iodine on the thyroids of 2,687 school children in Lincoln County, Nevada, and Washington County, Utah, two areas known to have received high levels of fallout. In Graham County, Arizona, 2,140 similar children were also studied as a comparison group. The screening of these school children by teams of three physicians was begun in Utah and Arizona in September of 1965, and in Nevada in 1966. The screening of the children was repeated in 1966 and 1967.

The examining physicians were divided into teams of three each and were sent into the communities being studied. The thyroid gland of each child in the study was examined independently by each member of the three-physician screening team for evidence of assymetry and/or nodularity. A questionnaire was completed for each child to obtain demographic data and a complete residence history. Any child who was suspected of having an abnormal thyroid gland by the screening team was referred to a panel of three experienced thyroidologists for evaluation.

Because the examining physicians knew the residence of the children that they were screening, an inherent bias was introduced into the study. However, an internal control was present in the study as the physicians didn't know the residence history of the children they were examining. Using the residence history and the activity readings recorded during and following the weapons testing, it was possible to calculate exposure history for each child in the study. The test series that were included in the study were Tumbler-Snapper, Upshot-Knothole, Teapot, Plumbob, and Hardtack II. These shot series occurred over the period of time from March 1952 to November 1958.

The gamma activity readings for each shot in these series were recorded by the radiological safety organization for populated and unpopulated areas within a 200-mile radius of the test site, and by the New York Operations Office for areas greater than 200 miles from the test site. The gamma exposure readings in mr/hr were decayed to 24 hours after each shot by using the Way-Wigner decay law. Next, the H + 24 hours exposure was converted to microcuries of iodine 131 per liter of milk using Tamplin's model (1). This measurement was calculated for each 30-day period for every area in the country where a child in the study was residing during this time. Assuming an average thyroid gland weight dependent on age for each child, the accumulated potential exposure in microcuries of iodine 131 per liter of milk per gram of thyroid tissue was calculated for each child. Exposures were accumulated for each child for the age period from six months preparturition to before the second birthday, up to the fifth birthday, and the total exposure from all the tests in the included series. The distribution of the children in the study by state of examination, exposure, and age of exposure is shown in Table 1. Using these exposure categories as the primary categorization of the children in the study, the distribution of abnormal glands according to other parameters of possible significance is being studied. A manuscript of these findings will be completed and submitted for publication early in 1971.

TABLE 1. POTENTIAL EXPOSURE FROM FALLOUT TO CHILDREN IN UTAH, ARIZONA, AND NEVADA

		μCi per Liter of Milk per Gram of Thyroid												
		0.0	0.25	0.50	0.75	1.00	1.50	2.00	3.00	4.00	5.00	7.00	9.00	Unknown
Exposure Before Age 2	Total	2280	1555	228	91	45	61	37	212	84	44	3	----	187
	Utah	1035	510	159	56	30	49	32	210	81	43	3	----	96
	Arizona	1127	890	13	10	5	1	1	1	--	--	--	----	82
	Nevada	118	145	56	25	10	11	4	1	3	1	--	----	9
Exposure Before Age 5	Total	563	2451	439	229	97	294	204	207	91	61	4	----	187
	Utah	258	557	322	180	68	274	195	204	87	60	3	----	96
	Arizona	277	1719	32	9	9	7	2	1	1	--	1	----	82
	Nevada	28	175	85	40	20	13	7	2	3	1	--	----	9
Total Exposure	Total	219	2633	488	243	117	293	247	223	112	61	4	----	187
	Utah	123	541	367	192	84	272	238	221	107	60	3	----	96
	Arizona	73	1920	34	9	10	7	2	---	2	--	1	----	82
	Nevada	23	172	87	42	23	14	7	2	3	1	--	----	9

Reference

1. Tamplin, A.R., and H.L. Fisher. 1966. Estimation of dosage to thyroids of children in the U.S. from nuclear tests conducted in Nevada during 1952 through 1955. TID-4500, UC-48. AEC Contract No. W-7405-eng-48.

C. RADIATION REGISTRY OF PHYSICIANS[1]

P. M. Hamilton

The registry was developed in 1960 as a vehicle for long-term follow-up of radiologists for the purpose of studying the biological effects of continuous occupational exposure to low dose levels of ionizing radiation. The data generated by the registry will provide information on both somatic and genetic effects of ionizing radiation.

Since July 1968, the operation of the registry has been a joint effort of the Bureau of Radiological Health, the American College of Radiology, the College of American Pathologists, and the National Academy of Sciences-National Research Council, the central registry unit being maintained at the Bureau of Radiological Health.

The original registry population was composed of the total membership of the American College of Radiology and the College of American Pathologists as of 1961. All foreign residents and persons reported deceased or resigned prior to January 1962 were excluded. New members have been added to the registry as they enter into the college. At present, the registry population is composed of 7,546 radiologists and 5,678 pathologists who are or have at one time been residents of the United States.

The basic source of data is a questionnaire which is mailed to all physicians as they are added to the registry. The questionnaire is intended to furnish information about personal characteristics of the respondent, professional career, description of exposure to radiation, reproductive history, personal and family history of disease, and exposure to other health hazards.

A major factor in the successful operation of the registry is getting each physician to return a completed questionnaire to the unit. Several steps are being taken to increase the number of questionnaires returned and the quality of information given by the physicians. The questionnaire is being revised to simplfy its completion and, at the same time, increase the quality of information given. Original queries are sent to new members soon after their entry into the college and re-queries are made to those physicians who have not responded within a few weeks. Since the initiation of these procedures, response rate on the initial mailing has increased from 49 percent to 61 percent.

A preliminary analysis of some descriptive data such as choice of specialty, smoking habits, and prior experience with tuberculosis, support the premise that pathologists can serve as an adequate comparison group for this study.

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[1] A full paper discussing this study, "Radiation Registry of Physicians," by C. Silverman and R. Seltser, has been accepted for publication in Radiology.

D. OVARIAN IRRADIATION

Paul J. Connors

During the period 1925-1960, a radiologist in New York City utilized low dosage exposure as a stimulus to the ovaries and pituitary of a series of females referred to him with documented sterility. The primary aim of this project is to survey the records available on these patients and investigate the results of this form of therapy. Almost 50 percent of the women became pregnant following radiation. Prevalence of miscarriage was high, but it did not approach that for pre-exposure pregnancies.

TABLE 1. REPRODUCTIVE HISTORY

Total Patients Studied	850
Pre-exposure Pregnancies	290
Pre-exposure Miscarriages	180
Pre-exposure Live Births	111
Patients Pregnant Postexposure	421
Postexposure Pregnancies	952
Postexposure Miscarriages	205
Postexposure Live Births	753
Postexposure Twins	6
Postexposure Males	375
Postexposure Females	378

Currently our attention is being directed to the presence of clinical markers of possible deleterious effects of this exposure in the first generation offspring. Gross review of the data indicates an apparent increased incidence of congenital anomalies in this group. These have been principally and most noticeably of the cardiovascular and central nervous systems (Table 2). These initial findings will be documented with further investigation.

We presently feel that this form of radiation has proven itself quite efficacious in the specific treatment of females with a history of documented, protracted sterility. However, we fear that this preconception form of radiation might have demonstrated harmful effects even in the first generation progeny.

TABLE 2. CONGENITAL ANOMALIES

Central Nervous System	
Malformation	8
Down's syndrome	2
Cardiovascular System	
Malformation	5
Ocular	
Malformation	1
"Deviated" eye	7
Genitourinary System	
Undescended testicle	3
Penis deformity	1
Multiple kidneys	1
Integument	
Hemangioma	3
Ear malformation	1
Miscellaneous (single)	
Pyloric stenosis	
Coeliac disease	
Polydactyl	
Imperforate anus	
Hirschspring's disease	
Disfigured hand & foot	

E. PARENTAL RADIATION EXPOSURE AND DOWN'S SYNDROME (MONGOLISM)

Charlotte Silverman, Project Officer

A principal purpose of the present study by the Johns Hopkins University is to test the suggestive previous finding that radar exposure in fathers may be associated with the occurrence of Down's syndrome in their offspring (1,2). This is being done by (1) collecting data on parental exposure and military service as well as other pertinent factors for a new and independent series of mongols and their matched controls, and (2) updating and checking information on military service and radar exposure of the original series. The original series included the parents of 216 mongoloid children and 216 control children; last year more than 140 cases and their controls had been identified for the new series.

Since then, more complete listings from several of the previously screened sources became available: A new listing for 1968 from the Johns Hopkins Medical Index and a more complete listing of births from the Baltimore City Health Department, obtained through the Department of Population and Family Health. As a consequence, further supplementary cases were obtained from the original and new sources. This increased previous estimates of time needed for all phases of the study: Diagnostic screening; interviews of parents; control matching, search, location, and interviewing of those parents; and data validation and tabulation.

As a result of this enlargement of the study interview series and the concomitant chromosome study series, each phase of the field work, data handling, and analysis has been extended; at least 6 months beyond the December 1970 termination date will be required to complete the project.

References

1. Sigler, A.T., A.M. Lilienfeld, B.H. Cohen, and J.E. Westlake. 1965. Radiation exposure in parents of children with mongolism (Down's syndrome). Bull. Johns Hopkins Hosp. 117:374-399.

2. Cohen, B.H., and A.M. Lilienfeld. 1970. The epidemiological study of mongolism in Baltimore. Ann. N.Y. Acad. Sci. 171:230-237.

F. ATOMIC BOMB CASUALTY COMMISSION

Charlotte Silverman, Project Officer

The Atomic Bomb Casualty Commission (ABCC) was established in Japan in 1947 at the request of the President of the United States to conduct long-term studies of delayed effects on the survivors of the atomic bombs detonated over Hiroshima and Nagasaki. It is now a cooperative research agency of the U.S. National Academy of Sciences - National Research Council and the Japanese National Institute of Health of the Ministry of Health and Welfare.

The U.S. Public Health Service has cooperated in this program for many years by the assignment of professional personnel. Since 1956, the Public Health Service has assigned over 50 medical officers to the ABCC on 2-year tours of duty and a number of the officers have extended their period of service. Assignments have been made to the departments of pathology, medicine, and clinical laboratory (cytogenetics) where PHS officers have contributed significantly to the research and operating programs of the ABCC and to the world literature on radiation effects. Five PHS physicians were assigned to the ABCC during the year.

In the pathology department, PHS officers participated in the following studies: Ten-year lung cancer autopsy experience (1961-70) including information on smoking; prostate carcinoma in autopsies; pathology of salivary gland tumors, in cooperation with the department of medicine; autopsy, surgical, and clinical diagnoses of various tumors including breast, uterus, rectum, colon, and bladder; cytogenetics of tissues obtained at autopsy; bacteriology of terminal pneumonia; immunoelectrophoresis of Australia antigen in autopsy serum; and autoradiography of autopsy tissues.

In the department of medicine, a protocol for the study of circulatory aging was developed and approved. The objectives are to determine whether A-bomb exposure has resulted in premature impairment of cardiovascular function and to correlate cardiovascular disease risk factors and clinical manifestations with measurements of function. There was continued participation in the tripartite study of cardiovascular disease in Japan. A study of immunologic competence of leukocytes from the exposed and nonexposed, involving both clinical and laboratory investigation, was continued.

G. THE EFFECTS OF EXPOSURE OF CHILDREN TO DIAGNOSTIC DOSES OF IODINE 131

Edythalena Tompkins

The association of exposure of the thyroid of young children to x ray with the subsequent development of thyroid neoplasms, malignant and benign, has been demonstrated in several studies. Likewise, 17 of the 19 children exposed to fallout on Rongelap Island who received an estimated 175 rads external gamma and 500-1,400 rads to the thyroid from radioactive iodines have developed thyroid lesions. In contrast, thyroid nodularity was not different in children who lived in southwestern Utah and Nevada during maximal periods of fallout (see p.18). It is uncertain whether this latter finding is the result of doses lower than the calculated values or whether the dose did not produce a biological effect. The primary difficulty with interpreting these findings is the lack of knowledge of actual dose delivered to the thyroid in the individuals studied. To resolve this problem, the presence of thyroid nodules in a series of children who had been given iodine 131 for diagnosis of thyroid function is being evaluated.

A study of the distributions of the age, time of exposure, diagnosis, and calculated doses delivered to the thyroid of 3,000 children exposed to diagnostic doses of iodine 131 in 12 different medical centers between 1946 and 1964 has been completed. Two-thirds of the children were diagnosed as having normal thyroid glands without the complication of other endocrine disease. Of the normal children exposed before 1955 who were under five, 47 percent were exposed to less than a calculated 50 rads delivered to the thyroid, but 16 percent had more than 200 rads with values exceeding 3,000 rads in some individuals.

This population will continue to be studied. The large range of doses involved and the long latent period which has already elapsed provides an opportunity to establish the shape of the dose-response curve for the development of thyroid neoplasms in children exposed to iodine 131 radiation.

H. THOROTRAST QUANTITIES AND WHOLE-BODY COUNTS

F. M. Hemphill, R. D. Hesselgren

Thorotrast, colloidal thorium dioxide, was commonly used from 1930 to 1950 as a contrast medium for diagnostic x-ray studies. It was injected intravascularly into the patient and has been strongly implicated as the cause of liver tumors in the recipients.

This study investigated the significance of (1) quantity of Thorotrast administered, (2) years since Thorotrast administration, and (3) age of patient at time of Thorotrast administration, in estimating values of body burden. The standard multiple regression analysis technique was used. The objective was to seek practicable procedures of approximating body burden values in patients with Thorotrast burdens without submitting them to whole-body counting.

From the data on 25 patients who had received Thorotrast 13 to 20 years prior to whole-body counting, with an average age of 36.6 years at the time of carotid artery injection, the following conclusion can be drawn.

a. "Quantity of Thorotrast Injected" produces an apparently satisfactory estimate of body burden scores for assessments applicable to stratification of patients for research studies aimed at dose-effect determinations, as shown in Figure 1.

b. Over ranges of the data studied, "age of patient at injection" and "years since injection" were found not to be significant variables in the body burden scores.

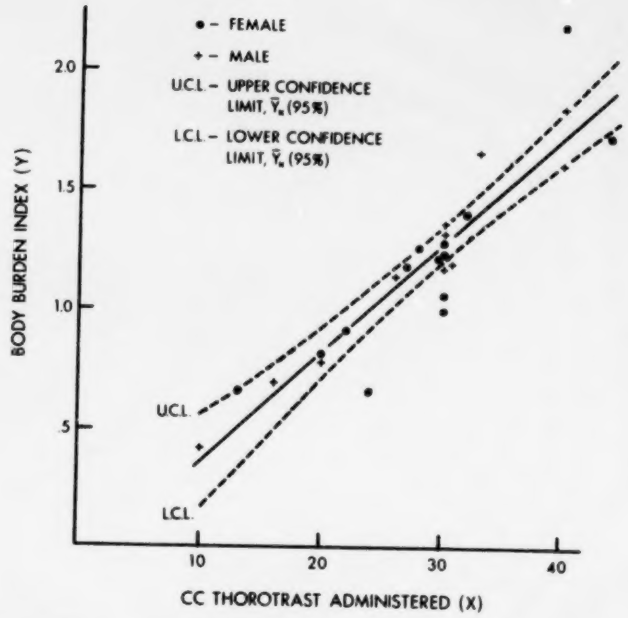

Figure 1. Patients by Body Burden Index and Volume (cc) of Thorotrast Administered.

I. FETAL IRRADIATION STUDY

Charlotte Silverman and Fay M. Hemphill, Project Officers

This long-term prospective study of x-ray pelvimetry conducted by Johns Hopkins University is concerned with the effects of in utero irradiation. The population comprises Negro females exposed during fetal life and their offspring. A study was made of the reproductive performance of exposed and nonexposed young women to determine whether an effect on fertility could be observed. The study is completed and an initial analysis of the data is presented.

The exposed mothers had higher birth rates than the controls, and similar rates of neonatal deaths and ascertained abortions (Table 1). Most comparisons of exposed vs. control second generation births failed to show a significant increase in problems encountered by exposed mothers in pregnancy and labor, or in the condition of their babies. However, when births were related to the time during fetal life (Table 2) when the initial exposure occurred, the following differences were observed: (1) Mothers exposed before 30 weeks of fetal life gave birth to significantly increased numbers of male babies and to fewer female babies compared with the mothers exposed later in fetal life or controls. These babies had longer gestations and lower Apgar scores. Placenta weights were lower and more mothers in this group had precipitate labors. (2) Babies born to mothers exposed at 37 or more weeks of gestation had significantly more neonatal jaundice.

The increase in birth rates is most marked in those exposed up to 34 weeks of gestation and is also elevated in mothers exposed late in gestation (40 or more weeks). By sex, a significant increase in male births and a decrease in female births occur in individuals exposed before 30 weeks of gestation. At 30-34 weeks and at more than 40 weeks the female rates are elevated and the male rates are relatively depressed. At 35-39 weeks equal numbers of both sexes are born (Table 2).

These data suggest that very low doses of x ray to the female fetus at certain times during gestation have an effect on her offspring. Individuals exposed at early ages have had more pregnancies, and the sex ratio of the offspring has been altered. Increased numbers of fetal deaths and abortions are also observed in the irradiated mothers.

One explanation for the increased number of pregnancies is that in humans certain doses of radiation are known to stimulate ovulation. Some evidence indicates that the radiation causes an acceleration of meiotic processes so that, although the number of eggs at early stages is sharply reduced, the number at later stages may temporarily equal or exceed that seen in unexposed controls.

It is more difficult to explain the observation that the irradiated mothers have given birth to almost twice as many males as females with a large increase in the male rates and an absolute decrease in the female rates. Most of the proposed hypotheses involve gross alterations in the chromosomal material or non-disjunction, and these possibilities can neither be affirmed nor eliminated by the current data. Buccal smears for sex chromatin analysis to determine non-disjunction of the maternal X chromosome showed that non-disjunction of the maternal sex chromosomes is not a prominent cause, if any, of the observed sex difference in these babies. However, the possibility of more subtle chromosomal alterations and mutuations has not been tested. The outstanding feature of these findings is that relatively low doses of radiation to the female fetus have a detectable effect on the second generation, although the mechanism of these changes is not known.

TABLE 1. F_2 BIRTHS TO MATCHED EXPOSED AND CONTROL F_1 MOTHERS
(All Births Ascertained Through 1968)

	Exposed	Control	P
Number in F_1 Population	1458	1458	
Total Pregnancies	834	700	.003
F_2 Live Births	784	673	.004
F_2 Fetal Deaths[a]	20	8	.024
F_2 Abortions[b]	30	19	NS
Twins	14	12	
Deaths--Neonatal and Later	29	32	
First Births	529	477	NS
Later Births	255	196	.004

a. The Baltimore City Health Department requires a certificate of fetal death for any fetus dead at birth if the gestation period is 20 or more weeks, or if the fetus weighs 400 or more grams.
b. Abortions are poorly ascertained by the study methods.

TABLE 2. F_2 BIRTHS BY WEEKS OF F_1 GESTATION AT TIME OF X RAY
(Births Through 1968 to Matched Population)

	Weeks of Gestation at Time of F_1 X ray								All Exposed[a]		Controls	
	30		30-34		35-39		40+					
	No.	Rate[b]	No.	Rate[b]	No.	Rate[b]	No.	Rate[b]	No.	Rate[b]	No.	Rate[b]
F_1 Population	111		231		634		294		1458		1458	
Live Births	68	61	136	59	303	48	159	54	784	54	673	46
Males (Live Births)	45	41	62	27	153	24	71	24	390	27	323	22
Females (Live Births)	23	21	74	32	150	24	88	30	393	27	349	24
1 Pregnancy	73	66	141	61	314	49	167	57	802	55	691	47

a. Includes exposed with gestation times unknown.
b. Rates are per 100 in F_1 population.

BEHAVIORAL STUDIES SECTION

Dr. A. K. Davis, Acting Chief

The Behavioral Studies Section was established to emphasize and encourage further investigations into mental and behavioral effects associated with radiation exposure. Mental illness reportedly increased in a group of persons who had x-ray treatments to the scalp for tinea capitis when they were children. Under Bureau auspices and P.L. 480 funds, tinea capitis patients in Israel who were similarly treated are being studied to evaluate and extend these findings. Clinical examinations of the subjects will be performed as well as electro-encephalographic examinations to detect minimal brain wave changes.

Nonionizing radiation has been shown to induce "nervous states" in experimental animals when they are placed in the direct beam. This evidence may indicate a direct effect on the behavior. Protocols to test this hypothesis are being formulated to examine behavior in radar or communication microwave fields.

A. DELAYED EFFECTS OF SCALP X IRRADIATION

Charlotte Silverman, Project Officer

This is a follow-up study of immigrant children in Israel who received x-ray treatments to the scalp for tinea capitis during the period 1949-1959. The once popular but now outmoded therapeutic procedure of x-ray epilation for ringworm of the scalp was used in a number of treatment centers in Israel. A course of treatment delivered an estimated 450-850 rads to the scalp (1) and an estimated 100-200 rads to the surface of the brain (2,3).

The objective of the study is to determine if late effects of such irradiation to the head can be demonstrated, particularly in the form of central nervous system changes or disorders, mental retardation, mental illness, and malignancies. The study was funded in June 1968 with P.L. 83-480 funds and is being conducted by the Department of Clinical Epidemiology of the Tel Hashomer Government Hospital in Israel. Dr. Baruch Modan, head of the department, is the principal investigator. Mr. Sheldon G. Levin, former statistician in our program who helped to develop the study, was on leave of absence to work on the project in Israel during the developmental phase.

The study plan specified a methodologic pretest prior to the full-scale study. During the pretest pilot year which was completed in 1969, methods were evaluated for identifying and locating irradiated patients, selecting and locating control subjects, determining interim morbidity and mortality experience, establishing the current health status of living subjects, and estimating radiation dose delivered to various parts and organs of the head and brain.

The decisions reached as a result of the pilot study established that: Two control groups would be used, population controls and sibling controls; the total study population would be approximately 30,000 comprised of about 11,000 irradiated tinea cases, 11,000 matched population controls,and 7,000 sibling controls; all cases and all controls would be traced and, to the extend possible, all records would be checked against hospital, tumor registry, welfare, mental hospital, police, school, and army records; and a sample of about 1,000 individuals, cases and controls, would have clinical examinations.

The full-scale study began in 1970. About 80 percent of the patients treated at the largest center in Haifa, where 8,000-10,000 were irradiated, have been traced; it is hoped that an additional ten percent can be positively identified. When controls are selected for these patients, tracing and follow-up of patients treated at the other centers will be started. The many difficulties encountered in corroborating study cases and completing identifications have delayed the selection of appropriate controls.

Plans are being made for clinical examinations of the treated and control sample groups. Standard or modified examinations, such as dermatological, neurological, psychological and laboratory examinations, will be performed by local consultant and project staff. Special electroencephalographic examinations, to detect evidence of minimal brain changes not discernible by standard clinical EEG examinations, are being arranged for with the assistance and participation of Dr. David R. Metcalf, Director of EEG Research, University of Colorado School of Medicine. It is hoped that the necessary equipment will be delivered early next year and that examinations can be scheduled after February 1971. It is estimated that 1 to 1½ years will be required to complete clinical examinations of the 1,000 study subjects.

Because of the size and complexity of the study, additional time, probably 1 to 2 years beyond the planned 3-year period ending in 1971, will be needed to complete the project.

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3. Werner, A., B. Modan, and S.G. Levin. A direct method of measuring total radiation dose to the brain, using the Fricke dosimeter. Presented at Symposium on New Developments in Physical and Biological Detectors, Vienna, Austria, November 1970.

DEMOGRAPHIC STUDIES SECTION
Dr. A. K. Davis, Acting Chief

The Demographic Studies Section is responsible for planning, execution, and analysis of studies of human populations exposed to ionizing and nonionizing radiation. These studies relate demographic and other relevant characteristics of the population to exposure parameters for specific sources. The section also identifies populations at high risk as a guide to studies of somatic and genetic effects, and integrates experimental laboratory findings with related population data.

Diagnostic radiation utilization is increasing in the 228 hospitals that were studied. Significant increases were found in specific radiation categories and multiple use on the same patients also increased. There was a significant increase in the number of patients receiving diagnostic radiation. To determine whether high levels of radioactivity had any observable short-term effects on mortality, several environmental radiation levels were related to the death rates in the area. None of the analyses produced any consistent association between changes in death rate and radiation levels of any of the variables.

A. RADIATION MORTALITY CORRELATION STUDY
Fay M. Hemphill, Frances B. Locke

Background

To determine whether high levels of radioactivity had any observable short-term effects on mortality, this study attempted to correlate environmental radiation levels with changes in death rates in areas where the radiation levels were measured. None of the analyses produced any consistent association between changes in death rate and radiation levels of any of the variables.

Although not the prime purpose of the study, correlations were made for each variable studied against each of the other variables. This analysis produced some interesting relationships, the significance of which are being further explored. The most interesting finding is that county iodine-131 readings in milk and in institutional diet are poorly correlated.

Methodology and Interpretation of Results

Environmental radionuclide levels were obtained from several sources including the complete collection of Radiological Health Data and Reports and the Health and Safety Laboratory Reports, water pollution reports, etc.

Eleven radiation variables were selected to be used for the study: Strontium 89 and 90, iodine 131, and cesium 137 in milk and in institutional diet; gross alpha and gross beta in water; and gross beta in air. The sources shown on Table 1 were the only ones that provided sequences of observation from 1960 to the present. Data for the study were used from stations that were fairly consistent in their measurements over the 7-year period from 1960 through 1966. Readings were low at the beginning and end of this period with high readings generally from 1962-1964.

TABLE 1. SOURCES OF RADIOACTIVITY MEASUREMENTS UTILIZED IN RADIATION MORTALITY CORRELATION STUDY

Source	Agency	Abbreviation	Medium	Radionuclide			
Pasteurized milk network	PHS	PMN	Milk	^{89}Sr	^{90}Sr	^{131}I	^{137}Cs
Institutional total diet sampling network	PHS	ITDSN	Diet	^{89}Sr	^{90}Sr	^{131}I	^{137}Cs
Radioactivity in surface waters of U.S.	PHS		Water	Gross Alpha		Gross Beta	
Radiation surveillance network	PHS	RSN	Air	Gross Beta			
National air sampling network	PHS	NASN	Air	Gross Beta[a]			

a. Converted to RSN values

Numbers of deaths were obtained by county for each year, and data for computing age-adjusted death rates were provided by the U.S. Census. In addition, for each county, demographic data were obtained indicating percent urban, percent non-white, median income, and population density.

The data were analyzed using multiple regression techniques with the death rate as the dependent variable, and the eleven radiation variables and four demographic variables as independent variables.

For the dependent variable, three measures of the change in age-adjusted death rate over the 7 years were used (resulting from regression against time for each county of the natural logarithm of age-adjusted death rates for each year). For each radiation variable, a county average for the years was used with logarithmic or arcsin transformations where advisable, to normalize the distributions. Counties varied as to the number of radiation variables with consistent readings, e.g., 59 counties had consistent milk readings, but only 19 had milk and diet, and only 10 had milk, diet, and air readings. Multiple regression analyses were prepared using various combinations of dependent variables with a measure of change in the age-adjusted death rate.

None of these analyses produced any consistent association between change in death rate and radiation levels of any of the variables. This is not conclusive evidence of no true correlation between health effects and radiation levels, since (1) mortality data are crude measures of health effects, (2) the data on radiation levels are not definitive measures of environmental radiation, and (3) health effects of high radiation levels may be long-term whereas this analysis attempted to measure only short-term effects.

Although not the prime purpose of the study, correlations were made for each variable against each of the other variables. Table 2 summarizes the analysis of correlations of radiologic readings in milk with institutional diet. Correlations are easily affected by extremes, therefore some rank correlations are presented.

TABLE 2. CORRELATION OF RADIOLOGIC READINGS BETWEEN MILK AND INSTITUTIONAL DIET YEARLY PAIRED READINGS
(Excluding Pairs if any Reading is Zero)

A. Correlations on all pairs possible

				^{131}I					
	^{90}Sr	^{89}Sr	^{137}Cs	All	Rank Corr.	Excl. 2 Extreme	1961 Only	1962 Only	1963 Only
N	148	122	131	49	49	47	10	18	19
r	.73	.83	.86	.13	.32	.28	.10	-.23	.36
Probability[a]	< .01	< .01	< .01	> .05	.03	> .05	> .05	>.05	> .05

a. Test of observed r against zero

B. Correlations using same counties and years

					Probability[a]		
N	^{90}Sr	^{89}Sr	^{137}Cs	^{131}I	^{90}Sr	^{89}Sr	^{137}Cs
47	.73	-	-	.28	< .01	-	-
42	.70	.66	-	.34	.02	.05	-
28	.76	-	.88	.32	.02	-	< .01
23	.68	.73	.85	.53	> .05	> .05	.04
23[b]	.59	.65	.79	.28	> .05	> .05	.02

a. Significance test of difference between ^{131}I and each variable
b. Rank correlation

Part A shows correlations for all paired readings available for each variable (excluding pairs where any reading was zero.) There are fewer pairs for iodine 131 than the other variables because iodine-131 readings were zero for either or both milk and diet in 1964 and 1965.

Part B shows correlations for radiologic data for the same counties and years. There were no cesium-137 readings in 1962, which explains no correlations for N=47 and N=42, and there were five instances where no strontium-89 readings were taken when other data were collected, thus explaining no correlations for N=47 and N=28.

In general, the correlations for strontium 89, 90 and cesium 137 are about 0.7 to 0.8, whereas the correlations for iodine 131 are about 0.3. Significance tests indicate that correlations for the former three nuclides are significantly higher than zero, whereas correlations for iodine 131 are not, and that the differences between correlations for iodine 131 and the others are often significant. Correlations of 0.7 and 0.8 indicate that the correlation explains 50 to 60 percent of the variation in readings, whereas the iodine-131 correlation of 0.3 explains about 10 percent of the variation in readings.

Readings for strontium 89 and 90 are highly correlated with each other, as are alpha and beta water readings. For demographic data, the percent urban and density values are highly correlated, as is age-adjusted death rate itself (rather than change in death rate) with percent non-white (Table 3).

TABLE 3. CORRELATIONS OF VARIABLES WITH EACH OTHER
(County Averages)

	Death Rate and Demographic Variables				
	Death Rate	% Non-white	% Urban	Median Income	Pop. Density
Death rate	+1.00	+ .52	+ .30	+ .18	+ .13
% Non-white		+1.00	+ .32	- .09	+ .43
% Urban			+1.00	+ .26	+ .68
Median income				+1.00	+ .30
Pop. density					+1.00
			Radionuclides		
Milk	^{89}Sr	^{90}Sr	^{131}I	^{137}Cs	
^{89}Sr	+1.00	+ .74	+ .27	- .05	
^{90}Sr		+1.00	+ .24	+ .40	
^{131}I			+1.00	- .03	
^{137}Cs				+1.00	
Diet	^{89}Sr	^{90}Sr	^{131}I	^{137}Cs	
^{89}Sr	+1.00	+ .78	+ .21	+ .19	
^{90}Sr		+1.00	- .05	+ .49	
^{131}I			+1.00	+ .01	
^{137}Cs				+1.00	
Water		Alpha	Beta		
Alpha		+1.00	+ .78		
Beta			+1.00		

B. DIAGNOSTIC RADIATION UTILIZATION IN SELECTED SHORT-TERM GENERAL HOSPITALS

Fay M. Hemphill, Frances B. Locke, and Rudolph D. Hesselgren

To estimate trends of medical radiation, two million records for the hospital stay of patients were sampled annually for 1963, 1966, and 1968 from over 200 short-term general hospitals. The records were studied for utilization patterns of ten diagnostic radiation categories.

There was a significant annual increase of discharged patients receiving any diagnostic radiation, and increased utilization of multiple categories per discharge. Significant changes were found in utilization of specific radiation categories, such as increase in "chest respiratory" and decrease in "survey chest." Patterns of utilization differed markedly by age, discharge diagnosis, and hospital size. After adjustment for hospital population changes, utilization of any diagnostic radiation showed increases of 1 percent annually.

The Commission on Professional Hospital Activities data comprising over two million patient hospital stay records annually from over 200 short-term general hospitals of the United States, were sampled in 1963, 1966, and 1968. The samples (about 80,000 per year) were studied by comparing utilization rates of ten diagnostic radiation categories (DRC) by year, size of hospital, age of patient, diagnosis, and frequency of conjoint use of two or more of the DRC.

There was a significant annual increase in both the proportion of patients who received diagnostic radiation and in the average number of the ten DRC received per hospital stay. After adjusting for distribution differences in hospital size, age, and diagnosis, the trend indicated an annual increase in rate of any radiation of 11 per 1,000 discharges.

The increase of rates over the years was true for all hospital sizes, with the smallest hospitals (<50 beds) showing the greatest increases (Figure 1). This is associated with the increase in average age of patients in small hospitals over these years.

The increase in rates was true for all age groups. The rates were lowest for births and highest for age 60+. Relatively, the births showed the largest increase in rates (Table 1, Figure 2).

Statistically significant increases occurred in 17 of the 20 diagnosis groups, with the exceptions being "congenital malformations" and "diseases of early infancy," which showed up and down patterns, and "deliveries" which had small increases. The lowest rates were in "births" and "deliveries," and highest in "symptoms" (Table 2).

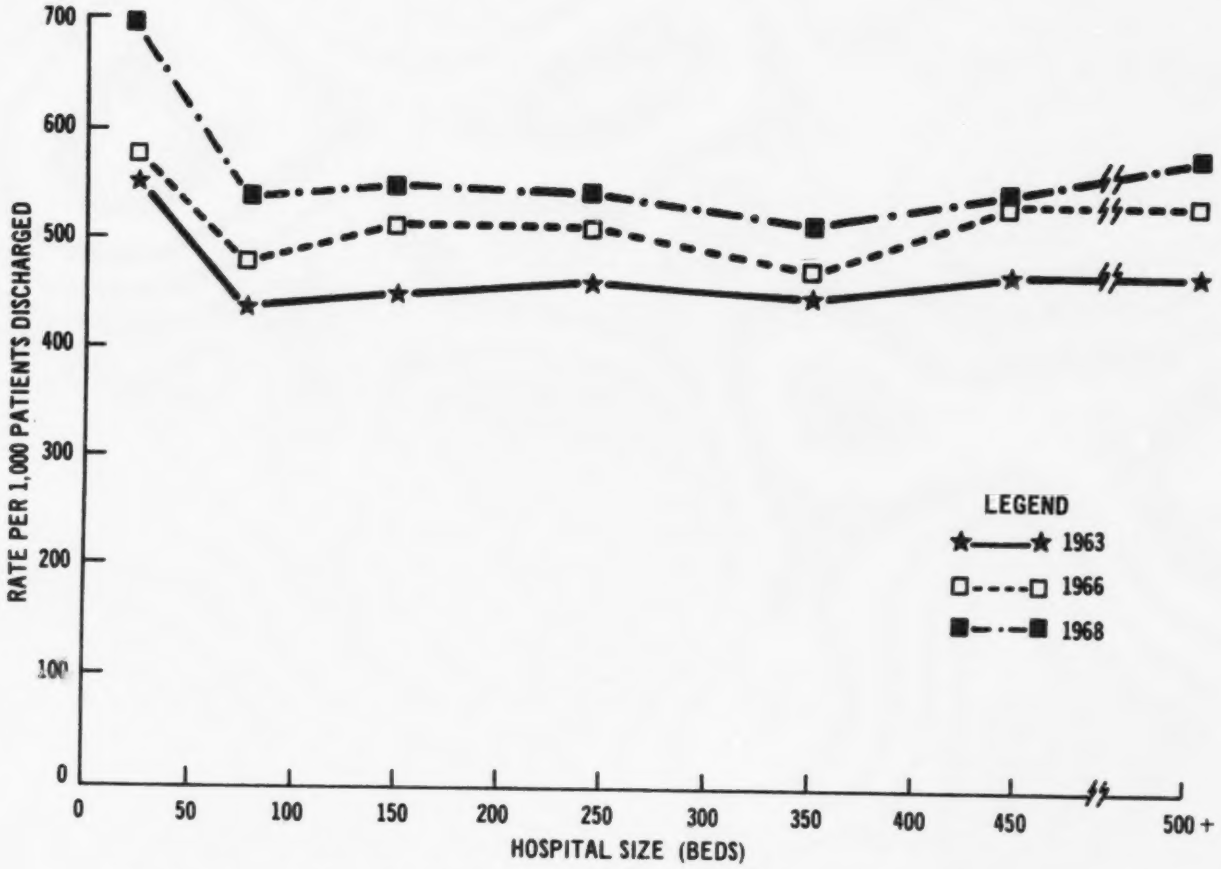

Figure 1. Rate of Any Diagnostic Radiation by Hospital Size; Selected Short-Term General Hospitals, U.S., 1963, 1966, 1968.

TABLE 1. RATES OF ANY DIAGNOSTIC RADIATION AND TOTAL DIAGNOSTIC RADIATION CATEGORIES ADMINISTERED BY AGE[a]

Age Group	1963			1966			1968		
	Number of Discharges	Rate/1000 Disch.		Number of Discharges	Rate/1000 Disch.		Number of Discharges	Rate/1000 Disch.	
		Any DRC	Total DRC Adm.		Any DRC	Total DRC Adm.		Any DRC	Total DRC Adm.
Births	11,122	29	33	9,125	36	44	9,882	44	53
15	12,404	404	511	10,855	460	618	11,171	493	660
15-29	17,840	348	476	16,646	389	575	19,225	401	612
30-44	14,748	523	812	12,467	563	967	13,401	595	1,051
45-59	12,446	697	1,173	11,916	725	1,313	13,483	758	1,435
60+	14,270	732	1,234	14,626	774	1,425	18,410	811	1,546
Total[b]	82,820	463	717	75,635	513	862	85,572	547	953

a. Selected short-term general hospitals, U.S., 1963, 1966, and 1968.

b. Sampling rate = 1:25 for 1963, 1968 and January-June 1966; 1:32 for July-December 1966.

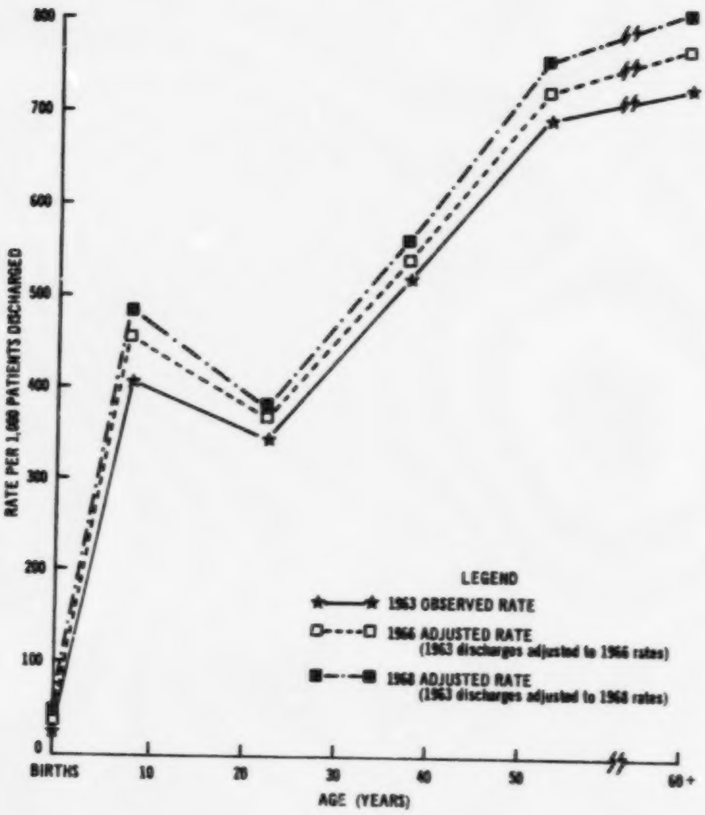

Figure 2. Specific Rates of Any Diagnostic Radiation Observed and Adjusted for Diagnosis; Selected Short-Term General Hospitals, U.S., 1963, 1966, 1968.

Significant increases occurred in 8 of the 10 categories (Table 3), with a significant decrement for "survey chest," and no change in "external soft tissue." The largest increases were in "chest respiratory" and "radioactive tracers." With the decreased use of "survey chest," the most frequently used category was "chest respiratory" followed by "skeletal" and "digestive." The least-used category was "external soft tissue." "Chest respiratory" plus "survey chest" accounted for over 50 percent of the total DRC.

The year-to-year trend of use of combinations of the ten DRC increased. About 15 specific combinations accounted for about 80 percent of all patients receiving any DRC, although these 15 varied from year to year.

TABLE 2. RATES OF ANY DIAGNOSTIC RADIATION AND TOTAL DIAGNOSTIC RADIATION CATEGORIES ADMINISTERED BY DIAGNOSIS[a]

Diagnosis	1963			1966			1968		
	Number of Discharges	Rate/1000 Disch.		Number of Discharges	Rate/1000 Disch.		Number of Discharges	Rate/1000 Disch.	
		Any DRC	Total DRC Adm.		Any DRC	Total DRC Adm.		Any DRC	Total DRC Adm.
Infective	688	621	946	643	663	1,075	685	698	1,149
Mal. Neop.	2,633	699	1,306	2,706	751	1,521	3,170	791	1,667
Ben. Neop.	2,351	469	724	2,185	530	842	2,483	559	944
Endoc. Syst.	1,765	698	1,190	1,743	760	1,412	2,052	777	1,523
Blood, etc.	391	673	1,325	387	724	1,561	449	742	1,619
Mental	1,312	581	951	1,431	604	1,089	1,654	655	1,180
Nervous Syst.	3,590	501	790	3,549	540	976	5,421	629	1,129
Circul. Syst.	6,009	656	1,045	5,924	722	1,235	6,185	786	1,424
Resp. Syst.	8,957	489	629	7,792	530	720	8,285	576	797
Digestive	9,995	627	1,091	9,528	675	1,274	10,318	697	1,349
Urogenital	6,681	609	993	6,466	637	1,091	7,732	666	1,173
Delivery, etc.	13,614	189	204	11,049	192	219	12,185	201	239
Skin, etc.	959	394	554	794	467	723	843	509	835
Bones, etc.	2,791	712	1,163	2,867	728	1,363	3,432	751	1,431
Cong. Malf.	665	618	970	681	639	1,010	677	614	1,021
Early Inf.	128	406	648	99	313	475	108	472	843
Symptoms, etc.	2,195	736	1,320	1,966	768	1,513	2,319	811	1,630
Injuries, etc.	6,456	730	1,011	6,185	753	1,118	7,125	768	1,221
Special	528	487	663	515	546	850	567	534	877
Births	11,112	29	33	9,125	36	44	9,882	44	53
Total[b]	82,820	463	717	75,635	513	862	85,572	547	953

a. Selected short-term general hospitals, U.S., 1963, 1966, 1968.

b. Sampling rate: 1:25 for 1963, 1968 and January-June 1966; 1:32 for July-December 1966.

TABLE 3. DIAGNOSTIC RADIATION CATEGORIES ADMINISTERED, BY YEAR[a]

Radiation Category	1963		1966		1968	
	Number	Rate/1,000 Discharges	Number	Rate/1,000 Discharges	Number	Rate/1,000 Discharges
Survey Chest	14,037	169	7,604	101	7,593	89
Rad. Tracers	828	10	2,154	28	3,521	41
Ext. Soft Tissue	176	2	185	2	223	3
Cardiovascular	721	9	1,021	13	1,209	14
Cent. Nerv. Syst.	927	11	936	12	1,216	14
Pleural and Perit.	2,518	30	2,793	37	3,538	41
Urogenital	5,909	71	6,248	83	7,556	88
Digestive	9,575	116	9,420	125	10,661	125
Skeletal	10,623	128	11,079	146	13,465	157
Chest, Resp.	14,063	170	23,778	314	32,575	381
Total Admin.	59,377	717	65,218	862	81,557	953
Total Discharges[b]	82,820		75,635		85,572	

a. Selected short-term general hospitals, U.S., 1963, 1966, 1968.

b. Sampling rate = 1:25 for 1963, 1968 and January-June 1966; 1:32 for July-December 1966.

C. DIAGNOSTIC RADIATION UTILIZATION IN 228 SHORT-TERM GENERAL HOSPITALS (UNITED STATES - 1963 AND 1966)

Fay M. Hemphill, Rudolph D. Hesselgren

To estimate trends of medical radiation, two million records for the hospital stay of patients were sampled annually for 1963 and 1966 from 228 short-term general hospitals. The records were studied for trends in utilization patterns of ten diagnostic radiation categories.

Patterns of utilization differed markedly by age and discharge diagnosis. There was a yearly observed increase of 17 per 1,000 discharged patients who received any diagnostic radiation. Although approximately 40 percent of the increase was attributed to changes in population distributions by age and diagnosis, and not to changes in physicians' practices, the remaining increment was significant.

Significant changes were found in utilization of specific radiation categories. There was increased utilization of multiple categories per discharge.

Data originating in the Professional Activity Study (PAS) of the Commission on Professional Hospital Activities during 1963 and 1966 from 228 short-term general hospitals of the United States were studied for patterns and changes in utilization of diagnostic radiation. Each year the study hospitals discharged more than 2,000,000 patients whose records were sampled to retrieve the study data. Rates per 1,000 discharged patients for each of ten diagnostic radiation categories (DRC) identified in PAS were compared by year, size of hospital, age of patient, diagnosis, and frequency of conjoint use of two or more of the ten DRC. Changes in distribution of the age and diagnosis groupings of patients (increase in average age, decline in births and deliveries) were found to be important in interpreting the observed changes in rates between the two years.

There was a marked relative increase in rate of "chest-respiratory" and radioactive tracers and a substantial decrease for "survey chest." Changes in observed patterns of the ten DRC between 1963 and 1966 tended to be uniform among size of hospital groups, age of patient, and diagnosis groups, i.e., the changes were not concentrated in a few of the sub-groupings. Table 1 shows rates by age and diagnostic radiation category for 1963 and 1966.

Two overall rates were given special consideration; any DRC, and total DRC administered. These rates varied together. The observed changes between years in these rates were found to be significant and meaningful. Overestimates of increase by some 42 percent are caused by shifts between the years in the percentage distribution of the hospital population in age and diagnosis and are not attributable to changes in physicians' practices. The residual remains statistically significant. The observed per year change in rate of 17 per 1,000 was determined to be more realistically 11 per 1,000.

TABLE 1. RATES BY AGE GROUP AND DIAGNOSTIC RADIATION CATEGORY[a]

Age Group	Number Discharges[c]	Rate[b] of Any Radiation	Rates[b] by Diagnostic Radiation Category (DRC)										
			Rate[b] of Total Radiation Categories Administered	Chest Respiratory (1)	Skeletal (2)	GI Tract (3)	Genito-Urinary (4)	Pleural & Peritoneal (5)	CNS & CNS Spaces (6)	Cardio-Vascular (7)	External Soft Tissue (8)	Radioactive Tracers (9)	Survey Chest (10)
1963													
TOTAL	82,820	463	717	170	128	116	71	30	11	9	2	10	169
Births	11,112	29	33	14	7	2	0	2	0	0	1	0	7
< 15	12,404	404	511	169	116	33	40	18	8	5	1	1	120
15-29	17,840	348	476	76	97	52	56	18	8	4	1	6	158
30-44	14,748	523	812	151	138	139	91	32	18	8	3	13	217
45-59	12,446	697	1,173	272	199	228	117	49	22	18	4	20	243
60 +	14,270	732	1,234	339	199	232	113	61	11	17	3	19	240
1966													
TOTAL	75,635[d]	513[e]	862[e]	314[e]	146[e]	125[e]	83[e]	37[e]	12[f]	13	2	28[e]	101[e]
Births	9,125	36	44	27	8	2	1	3	0	0	0	0	3
< 15	10,855	460	618	289	131	37	53	22	6	10	1	6	63
15-29	16,646	389	575	173	121	56	59	25	9	6	1	14	111
30-44	12,467	563	967	309	159	150	100	37	21	13	4	37	137
45-59	11,916	725	1,313	451	212	225	126	54	25	24	5	54	117
60 +	14,626	774	1,425	566	208	241	132	69	12	24	3	52	

a. Short-term general hospitals, U.S., 1963 and 1966.
b. Rate per 1,000 discharges.
c. January-December 1963 and January-June, 1966, sampled at 1:25; July-December 1966 sampled at 1:32.
d. Distributions of discharges, 1963 to 1966, differ significantly, $p < .01$.
e. Difference in rate, 1963 to 1966 significant, $p < .01$.
f. Difference in rate, 1963 to 1966 significant, $.01 < p < .05$.

The observed increase in rate of total DRC exceeded that of any DRC; its excess was accounted for, in large part, by increased utilization of two or more of the ten DRC during a patient's hospital stay (Table 2).

The data from the 228 hospitals when compared with an additional 511 similarly selected hospitals, indicated that the set of 228 did not show a unique pattern of DRC utilization. The beginning of Medicare, July 1966, apparently did not produce substantial changes in diagnostic radiation utilization within the study hospitals. Study of more years of data from the same set of 228 hospitals would add to reliability of the trend of diagnostic radiation utilization.

TABLE 2. DISCHARGES AND ADMINISTRATIONS BY NUMBER OF CATEGORIES IN COMBINATION[a]

Number of Categories in Combination	Discharges[b]				Administrations			
	1963		1966		1963		1966	
	Number	% of Total	Number[c]	% of Total	Number	% of Total	Number[c]	% of Total
0	22,157	53.3	20,742	48.7	0	0.0	0	0.0
1	11,841	28.5	12,052	28.3	11,841	39.6	12,054	33.0
2	5,288	12.7	6,216	14.6	10,576	35.4	12,432	34.0
3	1,731	4.2	2,520	5.9	5,193	17.4	7,560	20.7
4	435	1.0	825	1.9	1,740	5.8	3,300	9.0
5	90	0.2	191	0.4	450	1.5	955	2.6
6	14	< 0.1	38	0.1	84	0.3	228	0.6
7	3	< 0.1	5	< 0.1	21	0.1	35	0.1
8	0	0.0	0	0.0	0	0.0	0	0.0
9	0	0.0	1	< 0.1	0	0.0	9	< 0.1
10	0	0.0	0	0.0	0	0.0	0	0.0
Total	41,559	- -	42,590	- -	29,905	- -	36,573	- -

a. 228 Short-term general Hospitals, U.S. - Jan.-June 1963 and 1966.
b. Sampled at 1:25.
c. Distribution of discharges, 1963 to 1966 differ significantly, $p < .01$.

SOMATIC STUDIES SECTION

Dr. Loren F. Mills, Acting Chief

The Somatic Studies Section fosters and participates in studies of human populations exposed to nonionizing radiation. Radiation-related changes in human growth and development, changes in organ function, and changes in patterns of morbidity and mortality are investigated by the section. Data from the studies are integrated with experimental laboratory findings and related to population data.

To broaden the scope and increase the study activities relating to population exposure to nonionizing radiation, several new program areas are being developed. One will seek an association between exposure to nonionizing radiation and reasons for retirement for physical disability among U. S. Air Force personnel. A second project is a prospective-retrospective study to determine if cataracts or other lens opacities are associated with the therapeutic application of heat with microwave diathermy. The third project will attempt to identify and assess health risks from radar among occupationally exposed naval personnel and their offspring.

The Radiation Incidents Registry has processed data on 149 reported incidents and reported potential incidents involving approximately 200 persons. Eye and skin damage were the most frequently reported health effects from electronic product radiation. The most frequent form of radiation that produced the injuries was x ray. As yet, the Registry has received no reports involving sonic, infrasonic, or ultrasonic waves. In order to broaden the base of the Radiation Incidents Registry, tabular data and individual reports of radiation injury have been requested from the Bureau of Employees' Compensation and State Health Departments.

A. FOLLOW-UP STUDY OF RADAR WORKERS

Charlotte Silverman

Proposed Naval Study

The objective of this proposed study is to identify and assess health risks from microwave radiation (radar) among occupationally exposed naval personnel and their offspring.

The proposed approach will identify and follow up classes of former graduates of selected naval technical schools to determine whether these graduates who were exposed to microwave radiation during military assignments and in later civilian work differ from those not occupationally exposed, with regard to their mortality and morbidity experience, reproductive performance, and the health of their children.

The study will involve perhaps 20,000 men and is to be done by searching veterans' administrative and medical wartime military records, administering follow-up questionnaires, searching civilian records, and conducting household interviews if required.

Pilot work was started in 1970 under contract with the Department of the Navy to determine whether such a population-based prospective epidemiologic study is feasible. The National Academy of Sciences - National Research Council Followup Agency is participating. Three basic considerations are under review at this stage of planning: Sources of appropriate and adequate study groups of Navy-trained personnel, feasibility of estimating microwave (radar) exposure from military records, and problems involved in obtaining long-term morbidity information about the men and their families.

Proposed Radar Study, U. S. Air Force

Although the biological effects of microwaves have been studied, certain areas, such as the possibility of cumulative effects from subthreshold exposures, functional changes from low intensity irradiation as reported by the Russians, and possible nonthermal changes need further clarification. Large gaps also exist in the current knowledge of possible genetic implications and the actual long-term effects, if any, of microwave radiation on humans.

The basic purpose of this project is to determine if there is an association between a military occupational specialty and reasons for separations and retirements for physical disability among U. S. Air Force personnel. Of special interest are those occupational specialties that require the individual to be in or about radar or microwave fields or devices during the normal course of his duty.

Members of the population that we would like to examine would have all received disability discharges for the most recent year available. The data available are: (1) Discharge diagnosis and all secondary diagnoses, (2) time in service, (3) age, (4) sex, (5) Air Force Specialty Code (AFSC) number, (6) serial number, (7) cause of injury, and (8) underlying cause.

Initially, the frequency with which the various specialties appear within a given diagnostic category will be tabulated and analyzed. The results of these evaluations should identify future problem areas for investigations, and, in addition, help formulate standards that are suitable for civilian populations exposed to markedly lower levels.

B. RADIATION INCIDENTS REGISTRY

Loren F. Mills, Phyllis Segal

Background

The Radiation Control for Health and Safety Act of 1968 (Public Law 90-602) was enacted to protect the public from unnecessary exposure to radiation from electronic products. Although the increasing numbers of electronic devices which are appearing as consumer products and in the fields of medicine, industry, and communications exemplify scientific progress, they may also result in needless and adverse biological effects to the population. Under the Act, the Bureau of Radiological Health was given responsibility for conducting a radiation control program in which electronic products capable of emitting ionizing or nonionizing radiation, or sonic, infrasonic, or ultrasonic waves are subject to investigation and control.

The Division of Biological Effects has established the Radiation Incidents Registry as a means of appraising the incidence of biological injury and effects. Planning sessions were held to determine the most effective methods for data collection and analysis, and in December 1969 the Radiation Incidents Registry began operation.

As a means of soliciting data and explaining the purpose and nature of the registry, about 4,500 placards with covering letters were sent to people who would be likely to have such information.

Discussion

The following criteria have been established for reporting radiation incidents:

(1) Instances in which a person (or persons) was known to be exposed to radiation in excess of permissive limits, and a biological effect was reported.

(2) Unexpected injuries, latent health effects, or deaths attributed to radiation exposure.

(3) Potentially injurious situations where: (a) the person lacks the knowledge necessary for the safe operation of a radiation-producing device and (b) unknown or excessive amounts of radiation were emitted from the device, but no biological effect was noted.

Reports of radiation incidents which have appeared in the professional literature are accepted in the registry and regarded as public knowledge. No further inquiry is made regarding their validity. However, this does not constitute the registry's acceptance of a cause and effect relationship.

Findings

Data have been processed on 209 reported incidents and potential incidents involving approximately 276 persons. Seventy-six of these incidents were taken from a report by Thomas J. O'Toole, "Studies in Workmen's Compensation and Radiation Injury," Vol. II, the Department of Labor and the Atomic Energy Commission, U. S. Government Printing Office (1965) 0-786-463. Table 1 shows the types of exposure and selected characteristics of incidents reported to the registry as of December, 1970, and Table 2 shows this information for the workmen's compensation and radiation injury claims. Eye and skin damage were the most frequently reported health effects due to electronic product radiation.

TABLE 1. TYPES OF EXPOSURE AND SELECTED CHARACTERISTICS OF INCIDENTS REPORTED TO THE RADIATION INCIDENTS REGISTRY AS OF DECEMBER 1970 (Excluding Workmen's Compensation Claims)

Type of Exposure	Number of Incidents	Number of Persons		Type of Injury[a]			
		Total	Injured	Eye	Skin	Blood	Other
All Claims	133	192	141	85	58	5	9
Occupational							
Nonionizing	71	84	77	64	12	2	7
Ionizing, x ray	47	69	38	7	34	3	1
Nonoccupational							
Nonionizing	7	16	14	14	1		
Ionizing, x ray	7	22	11		11		
Ultrasound	1	1	1				1

a. Some persons had more than one injury.

Reports have been collected from 31 states, three foreign countries, and the Federal Bureau of Employees' Compensation. Approximately 210 persons were reportedly exposed prior to October 1968, when P.L. 90-602 was enacted, and 66 have been reportedly exposed since that time. There were no reports of children under age 16 being exposed. Between the ages of 16 and 30, 57 individuals were reportedly exposed; between 31 and 44, 55 individuals were involved; and over age 45, 36 individuals were involved. Age data on the remaining individuals were not available.

TABLE 2. TYPES OF EXPOSURE AND SELECTED CHARACTERISTICS OF WORKMEN'S COMPENSATION AND RADIATION INJURY CLAIMS INCLUDED IN RADIATION INCIDENTS REGISTRY AS OF DECEMBER 1970

Type of Exposure	Number of Incidents	Persons Claiming Injury	Type of Injury[a]			
			Eye	Skin	Blood	Other
All Claims	76	84	21	22	16	35
Workmen's Comp. and Bureau of Employees' Comp.						
Nonionizing	12	12	9		2	4
Ionizing	39	39	8	5	14	17
Both	3	3	3			
Unknown	1	1				2
Company						
Ionizing	7	16	1	9		6
Unknown	1					1
Common Law, X Ray	13	13		8		5

a. Some persons had more than one injury.

The reports specified exposure to 200 males, 31 females, and 45 persons whose sex was not reported. Of the incidents reported, persons were exposed in the following decreasing order by occupation: Technicians in the fields of engineering, physical science, and miscellaneous professional services, persons employed in electrical equipment industries, hospital personnel, and students performing experimental work in educational institutions. Figure 1 shows the percentages of persons reportedly exposed to ionizing and nonionizing radiation. There has been one report involving ultrasonic energy.

The first Registry publication was "Radiation Incidents Registry Notes", October 1970. "Notes" describes in brief the methods used by the Registry to collect and analyze reported data and presents a small sample of the types of incidents that have been reported to the registry. "Notes" will be updated and distributed about three times a year. The "Radiation Incidents Registry Report 1970" (BRH/DBE 70-6) is being prepared and will be distributed as a Division of Biological Effects technical report.

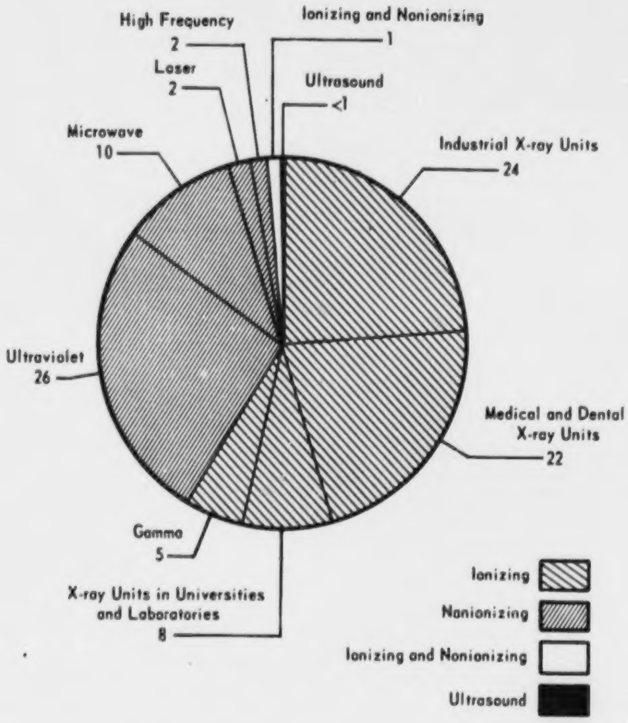

Figure 1. Percentage of Persons Reportedly Exposed to Ionizing and Nonionizing Radiation and Ultrasound; Radiation Incidents Registry, as of December 1970.

C. BIOLOGICAL EFFECTS OF DIATHERMY

Loren F. Mills

The purpose of this project is to determine the biologic effects associated with the therapeutic application of heat by means of microwave diathermy. The need for this information is based on the widespread use of diathermy in medical and dental practice and the probable association between microwave radiation and cataract formation. Therefore, by determining the frequency of cataracts or other lens opacities in a treated population, this study will evaluate the biologic effects associated with acute exposure and repeated exposure to therapeutic microwave diathermy.

Persons previously treated with microwave diathermy, with or without prior eye examination records, will be recalled for a thorough clinical appraisal of the eyes, ears, nose, and oral cavity (head and neck physical). The lens of the eye will be examined utilizing the slit-lamp technique. Persons about to be given heat treatments with microwave diathermy will also be given the same head and neck physical examination and then recalled periodically at a later date. Efforts will be made to reduce examiner bias, i.e., patients will not be identified by group, prospective or retrospective, at the time of examination. The physical examination data will be tabulated and analyzed employing appropriate statistical procedures.

At present there are three known exposed populations available for study. All of the people, in each population, have had therapeutic diathermy treatments with microwave radiation at the 2450 MHz range.

(1) The Oral Surgery Department, Clinical Center, National Institute of Dental Research, has been utilizing microwave diathermy, following the extraction of teeth, for approximately two years. Each therapeutic diathermy procedure lasts from five to eight minutes and has a power density range between 150 mW/cm^2 and 200 mW/cm^2. The emitting device is usually held by the patient at a distance no greater than one inch from the surface over the angle of the mandible. No more than three such treatments are given. Usually one or two are sufficient following tooth extraction.

The population consists mainly of individuals used as "controls" for one or more of the other institutes at the National Institutes of Health, e.g., controls for the Heart or Cancer Institutes. These persons are young, under 30 years of age, usually college students, and in excellent health. There are fewer than 100 of them and they would make up a short acute exposure population.

(2) There are several clinics throughout the U. S. that utilize microwave diathermy for the therapeutic heating of the temporomandibular joint in cases of arthritis and traumatic injury. Each therapeutic procedure lasts from 15 to 20 minutes and has a power density range between 150 mW/cm^2 and 200 mW/cm^2. The emitting device is usually held by the patient at a distance no greater than one inch from the surface over the region of the temporomandibular joint. The number of heat treatments varies, so that a person might return once a week for a year. These populations are mostly older persons and their general state of health ranges from fair to poor. These individuals would constitute an acute long-term exposure group.

(3) A large number of naval personnel have been treated with microwave diathermy for post-immobilization trismus following fracture, reduction of the maxillo-mandibular facial complex, and other surgical procedures. The therapeutic diathermy procedure lasts from 15 to 20 minutes and has a power density range between 150 mW/cm^2 and 200 mW/cm^2. The area irradiated is the bilateral buccinator-masseter-internal pterygoid muscle complex. The procedure is either done by the therapist or by the patient under supervision. Usually four or five treatments are sufficient.

These individuals are young, usually under 25 years of age, and in fair health. The exact number available for study is not known; however, a good estimate would be 200. This population would constitute an acute intermediate exposure group. This study could be done on a collaborative basis.

The duration of the study is another important factor to be considered. Since the length of time required for cumulative and latent bio-effects to appear is not known, the study duration period of these three populations should therefore be on an "open-end" basis.

EXPERIMENTAL STUDIES BRANCH
Dr. Moris L. Shore, Chief

The Experimental Studies Branch plans, conducts, and supports an experimental and multidisciplinary research program including supportive dosimetry studies, to determine the biological effects of ionizing and nonionizing radiation. It provides experimental radiobiology support to all other Bureau divisions and maintains liaison with pertinent national and international organizations.

The Office of the Chief provides scientific direction to, and coordinates the activities of the six sections which comprise the Experimental Studies Branch. It additionally provides, through the Animal Care Unit, services to the entire staff of the Branch.

During this past year we have expanded our efforts, through reprogramming, in the area of microwave bioeffects investigation, and have initiated a study of the biological effects of ultrasound. There has been continued effort to investigate the biological effects of ionizing radiation including both 250 and 30 kVcp x rays as well as strontium-89 and tritium. The third in a continuing series of reviews of the scientific literature has been published as a Bureau report. This latest report covers ultraviolet radiation hazards. A fourth report is currently under editorial review and will examine the published literature on the biological effects of ultrasound. Previous reports have covered microwaves and lasers. The Branch has actively supported the development of electronic products standards--particularly the development and defense of the scientific basis for the microwave oven emission standard, published on October 6, 1970, in the Federal Register.

Studies which were undertaken to investigate the comparative biological effects of 30 kVcp x rays vs. 250 kVcp x rays have been concluded. These have shown that the low-energy x rays similar in spectral characteristics to those emitted from color TV units are, on a rad for rad basis, as effective in producing biological changes as are 250 kVcp x rays. It is not anticipated at this time that new comparative studies will be undertaken. The low-energy x-ray unit will continue to be used in laboratory studies, however, since it offers the unique availability of high dose rate, useful in our viral transformation studies.

Continuing attention is being devoted to the age sensitivity problem, including studies of in utero radiation to define critical periods of development when radiation may be particularly effective in producing deleterious effects. Our long-term study in cats dosed with strontium-89 is a significant factor in this program. Additionally, studies which have focused on protein synthesis in the fetus following in utero irradiation are being expanded to examine biochemical processes in the nervous system of animals exposed either to x rays or microwave radiation. These studies will simultaneously examine ultrastructural changes in the nervous system produced by exposure to electromagnetic radiation. Pilot studies are also under way, in collaboration with the National Institutes of Health to examine the effect of x rays and microwave radiation on neurochemical processes, specifically neurotransmitters, following both prenatal and postnatal electromagnetic radiation exposure.

Our research program on the neurophysiological effects of microwave radiation has been facilitated by the recruitment of three experimental psychologists, and a major acquisition of behavioral and EEG equipment from the Department of Defense. Final development of our neurophysiological facility, and the training of rhesus monkeys for behavioral studies is currently under way. This area of investigation is crucial, in view of our commitments under P.L. 90-602 and the current controversy related to the subtle effects of microwave radiation reported by Soviet scientists. It is of particular concern to us that recommended levels of exposure for microwaves in this country are 1,000 times higher than those permitted in Russia. It would be imperative, for the protection of public health and safety, that we examine critically the scientific basis for the Russian microwave standard.

Major progress has been made towards the development of a controlled environment-data acquisition facility to be used in connection with our studies on the biological effects of microwave radiation. This facility includes a lucite spherical environmental chamber, which has been engineered and is nearing final assembly at the National Institutes of Health. Within this chamber environmental factors, including atmosphere composition, temperature, humidity and wind velocity will be controlled precisely. A controller, in effect a mini-computer, will regulate the microwave radiation source, the environment, and will collect, analyze and store physiological data obtained during experimental runs. All elements in the system will be controllable during the experiment as a function of biological response or other predetermined and preprogramed experimental requirements. We are currently awaiting delivery of the environmental chamber, the random access disc memory, and a digital magnetic tape transport to complete the facility. Programs necessary for the experimental operation of the digital controller, including the digital-analogue, and analogue-digital converters are under development and are being tested.

The P.L. 83-480 Foreign Cureency Award Program is presently being used to fund research on the effects of x radiation on the central nervous system at the University of Sarajevo, Yugoslavia. This study is nearing completion of its first year and a report will be due in February 1971. Preliminary efforts are under way to establish a second study in Israel at the Weizmann Institute to examine the induction of virus by radiation from transformed cells. Such a process, if demonstrated, would have far-ranging implications in the therapy of tumors by radiation.

While current levels of budgetary support do not permit it, expanded levels of research effort will be required to implement the provisions of P.L. 90-602. Intramural competence and productive research will be a necessary ingredient in such expanded effort. However, it is clear that extramural research will have to play a significant role in developing information necessary to support reasonable performance standards to protect public health and safety. Our staff has been active in the identification of competence and interest, within the scientific community, for research in this area. We recognize the need for that balanced program of intramural and extramural research that will permit effective implementation of the provisions of P.L. 90-602.

ANIMAL CARE UNIT

Mr. Clayton F. Cisar, Chief

The Animal Care Unit provides animals and animal care for the studies conducted by the Division of Biological Effects at the Rockville, Maryland, laboratory. The unit assists the investigators in planning the space, selecting the most suitable animal species or strain, and providing the special breeding or handling required for a particular study.

One part of the Animal Care Unit provides animals for relatively short-term studies; the other part, operated by contract, cares for animals in long-term experiments. During the year, the unit bred and raised about 6,000 Chinese hamsters, and cared for approximately 3,000 rats, 500 mice, 30 swine, 10 rabbits, 30 guinea pigs, and a few cats and monkeys.

The general health of the animals has been excellent, owing to the strict hygienic discipline maintained in the colony by the animal caretakers. Good animal health is mandatory because the subtle effects occurring in irradiated animals may be overshadowed by ill health.

The Animal Care Unit assisted in the planning and execution of many research projects. Not the least of these projects this year involved the use of swine in a long-term, 18-month study. These swine were examined periodically for optical lesions and this necessitated the restraint of animals weighing more than 600 pounds. These animals were also sacrificed at the termination of the experiment by Animal Care Unit personnel to obtain tissue for histological examination.

DOSIMETRIC STUDIES SECTION
Mr. George Anderson, Chief

Responsibility of the Dosimetric Studies Section is two-fold; that of maintaining radiation facilities and related dosimetry services for research projects conducted within the Division of Biological Effects, and that of conducting associated research projects with primary emphasis on measuring and understanding the mechanisms of energy absorption in biological specimens. These projects relate directly to the energy delivered to and absorbed by man from the various electromagnetic radiation sources in his environment.

The section also provides technical support to the program in areas of bio-electronics, radiation physics and facility development. The staff includes five physicists, two electronics engineers, two instrument/equipment specialists, one electronics technician, two physical science aides and a secretary.

A. IONIZING RADIATION DOSIMETRY AND IRRADIATION SERVICE

Irradiation Services
James W. Rolofson, Wah Lee, Lynda D. Kramer

Providing irradiation services and dosimetric support to the Division of Biological Effects continued to be one of the primary functions of the Dosimetric Studies Section during 1970. From December 5, 1969, through November 24, 1970, irradiations were performed on 847 mice, 394 rats, 373 Chinese hamsters and 370 biological specimens, such as cell cultures, using the various radiation sources under our responsibility.[1] In addition, a total of 580 thermoluminescent dosimeter (TLD) rods were irradiated, as a service to the Division of Electronic Products, to exposures as large as 1.2×10^6 R.

Examples of typical dosimetric support furnished during the year ranged from designing and constructing a device allowing uniform radiation exposure of all fetuses in a 400-gram rat to minimizing attenuation differences while equalizing other variables for absorbed doses delivered to flasks containing hemopoietic cells irradiated with 30 kV and 250 kV constant potential x rays. A new tube installed in the medium energy therapy x-ray machine required a complete recalibration which also required a revision of all animal and cell culture primary dosimetry.

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[1] These numbers exclude the numbers of biological specimens shammed with the radiation facilities.

Thermoluminescent Dosimetry (TLD) Applications

Wah Lee, James W. Rolofson

Introduction

The phenomenon of thermoluminescence in some materials after exposure to ionizing radiation is due to the capture of an ionized valence electron at the site of a crystal lattice defect such as an impurity within the crystal. The captured electron is in a metastable state. When sufficient thermal energy is applied to the material, the metastable valence electron returns to its ground state, and a photon is emitted. Many thermoluminescent (TL) materials have been tested for application to ionizing radiation dosimetry. At the present, the most widely used TL material is lithium fluoride (LiF) of which TLD-100 has been the most widely accepted. Some of the outstanding dosimetric characteristics of LiF are:

1. It can be made into any size and shape and incorporated into any system for absorbed dose determination.

2. It has a wide range of dose response (from several mR to about 10^5 R).

3. It has a linear response from several mR up to about 500 R, and it is essentially dose rate independent (at least up to approximately 10^{10} R per second).

4. It retains its stored signal quite well. (It fades approximately 3% after a week's storage.)

5. It has a relatively low energy dependence. (It varies only 25% within the range of 20 keV to about 1 MeV effective photon energies.)

6. It is approximately tissue equivalent (~20% higher in mass energy absorption coefficient).

7. It is simple to handle and is reusable.

Equipment

The Dosimetric Studies Section presently has two TLD readers. One (Madison TL-5) is used for routine dosimetry; the other (laboratory built) is primarily devoted to research within the section.

Secondary standard exposures are measured with Victoreen thimble and end window chambers. All chambers are read with a Victoreen

Model 570 R-meter. The chamber-electrometer sets were calibrated against free-air ionization chambers at the National Bureau of Standards with various radiation beam qualities. The thimble chambers were rated with an accuracy of ±2% within the range of 30-400 keV effective photon energies; the end window chambers were rated with a ±10% accuracy within the effective photon energies of 6-35 keV. However, the precision of exposure measurement can be reproduced within ±1% with all the above chamber-electrometer sets at a given x-ray beam quality. Therefore, by knowing the calibration factor of the chamber at a given radiation quality, ±2% accuracy of exposure measurements can be achieved with all chambers.

There are three types of TL materials being used at this laboratory: LiF (TLD-100), CaF_2, and $CaSO_4:Dy$. LiF is used exclusively for routine dosimetry. Powder LiF has been found to be the most versatile and is used in this laboratory for radiobiological dosimetry. Occasionally extruded LiF rods 0.5-1.0 mm in diameter are used for implantation in small animal organs. Also teflon discs, 0.5 mm thick, impregnated with LiF have been used to measure interface doses. In general, different dosimetric problems require different sizes and shapes of dosimeters and methods of approach.

Characteristics of LiF Dosimeters

With practice and experience in methodology, dosimetry using LiF can be reproduced within ±1% or less. Factors such as annealing, exposing, and readout procedures can affect its reproducibility, therefore operational procedures involving TLD must be consistent.

One of the outstanding dosimetric characteristics of LiF is its relatively low energy dependence. For instance, at 20 keV effective photon energy, LiF's response is only 25 percent higher than that at 662 keV photon energy. The authors have observed that for an x-ray beam with a given kVp and half-value thickness (HVT), if the HVT is arbitrarily changed by less than a factor of two by added filtration, the energy responses of LiF vary within ±3%. It is therefore possible to utilize a primary x-ray beam to approximate the radiation qualities in phantoms of small animals, such as rats and guinea pigs, in calibrating the LiF dosimeters without appreciable error. In the event there is an appreciable attenuation of the primary beam in the phantom, the in-phantom radiation quality at the site of interest can be determined using one of the methods described below, and using by necessity a similar x-ray beam to calibrate the LiF dosimeters. The energy dependence of LiF becomes especially important at lower photon energies, i.e., below 30 keV.

Radiation qualities inside the phantom can be determined by calibrating the dosimeters in air with a series of known x-ray beam qualities. The ratio of dosimeter responses in the phantom can be correlated to a known quality of x-ray beam in air with one of the two following methods:

(1) By using the ratio of relative responses of two different TLD materials with widely different energy responses (e.g., $CaSO_4$:Dy and LiF) or (2) by using the ratio of relative responses of one TLD material when it is encapsulated in a uniform thickness of filter material such as copper, to its relative response when not encapsulated.

As mentioned previously, consistency in all operational procedures is essential in thermoluminescent dosimetry. The following operational procedures adapted for LiF in this laboratory are quite satisfactory for radiobiological research dosimetry.

(1) Annealing

(a) Powder

After the phosphor has been used, stack a 100 mesh screen atop a 200 mesh screen and sift the powder through the 100 mesh screen. Wash the powder on the 200 mesh screen first with 95% ethyl alcohol, then with distilled water. (If the powder is clean, this step can be omitted.) Dry the powder over a heat lamp or in a dust free oven. After the powder is thoroughly dry and granular, put it in a Pyrex beaker and cover it with aluminum foil. Anneal it for 30 minutes at 400° C (±5° C). Let it cool to room temperature in a good heat conducting metal block. Then, anneal the powder for 24 hours at 80° C.

(b) Extruded Crystals

If cleaning is needed, wipe the crystals with alcohol followed by distilled water. Anneal them at 400° C for 15 minutes. Let cool to room temperature, then put them in a 100° C oven for 2 hours.

(c) Teflon Dosimeters

Cleaning procedure is same as that for the extruded crystals. Anneal the dosimeters at 300° C for 15 minutes. Let cool to room temperature and anneal them for 2 hours at 100° C.

(2) Exposure Standards

Dose measurement with LiF is relative to its response at a given standardized exposure. After obtaining the air exposure rate from a given x-ray beam quality with an appropriate Victoreen ionization chamber, LiF dosimeters can be suspended in air on masking tape and irradiated to a desired exposure. When exposing the standards, care must be taken to insure that all the phosphor is homogeneously irradiated. Homogenous dosing requires that the phosphor be thin enough so there is only negligible attenuation throughout the sample and that all the phosphor must be at electronic equilibrium condition. In order to achieve electronic equilibrium the phosphor must be covered with a shielding material of an appropriate thickness to exclude all the secondary electrons produced in air by the primary radiation photon spectrum. At the same time, the shielding thickness must be thin enough so there is negligible attenuation to the primary radiation spectrum. With photon energy less than 250 keV, a mass thickness of approximately 10 mg/cm^2 is usually sufficient for the shielding thickness. A mass thickness of 10 mg/cm^2 can be obtained with a thin sheet of plastic from commercial plastic bags. If shielding from water or other liquid medium is desired, the LiF powder can be heat sealed in the plastic. If a sufficiently hard x-ray beam is used, the phosphor can be encapsulated in a gelatin capsule (such as those used in the pharmacy for drug encapsulation). For small extruded rods (~1 mm in diameter) small diameter electrical wire insulation tubing can be used for encapsulation. Encapsulated extruded rods used for implantation in an animal can be sealed at the ends of the tubing with a water repellent putty.

(3) Read-out

Before reading out the exposed phosphor, anneal the dosimeters at 100° C for 5 minutes. This procedure erases all of the low energy trappings which fade rapidly at room temperature. Readout the phosphor at a heating pan temperature of 240° C (with the phosphor on the pan). During the read-out cycle, purified nitrogen gas at a flow rate of 1-2 ft^3/hr should be used to replace the air in the heating pan chamber. This procedure greatly reduces the amount of non-radiation induced thermoluminescence. An infrared filter may also be helpful in reducing the background signal from the heating pan. The amount of phosphor to be used should be just enough to cover the entire area of the heating pan (approximately 35 mg for a heating pan area of approximately 1 cm^2). An unknown exposure can be obtained by calculating its TL response per mg of phosphorus and comparing it with a known standard (TL response/mg/R).

Phantoms

In phantom dosimetry for radiobiological purposes the phantom material must be tissue equivalent. A material is said to be tissue equivalent if its mass energy absorption coefficients and mass density are equivalent to those of tissue over the range of photon energies under consideration. Many materials can be used for phantom dosimetry if their mass energy absorption coefficients and density are known and corrected to those of tissue.

In this laboratory Mix-D, a mixture of paraffin, polyethylene, magnesium oxide, and titanium dioxide, is commonly used for tissue equivalent material. In using Mix-D for phantom construction, a negative mold of the object to be made is cast in plaster of Paris and melted Mix-D is poured into the mold. Mix-D should be gently and constantly stirred when heating, and poured slowly when it is sufficiently cool to prevent bubble formation. If a non-perishable positive mold of an object needs to be made first (such as a brain from a skull cavity) Silastic rubber can be used.

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B. SPECTROMETRY

Wah Lee

Characterization of Radiation Spectra and Tissue Dose Distribution

In this study, low to medium energy x-ray spectra with various combinations of kVp's and added filtrations are examined with a high resolution detector, Ge(Li), and a 1600 channel analyzer. Spectral characteristics are correlated with the conventional specifications of an x-ray spectrum such as kVp, HVT, homogeneity coefficient, and depth dose function. An attempt will be made to qualify the term "effective energy" of an x-ray spectrum in terms of the ratio of energy responses of two different TLD materials ($CaSO_4$:Dy and LiF) and depth dose function in water. The results of this study will provide a basis for (1) simulating photons from any low- to medium-energy x-ray source, such as those emitted by electronic products, with an x-ray machine to irradiate biological specimens under convenient laboratory controlled conditions (the simulation of color TV emissions is the most recent example), (2) providing a source for standard exposure measurements, especially for dosimeters with strong energy dependence, (3) using a computer for dose calculation.

Detector and Spectrometer Calibration

An Ortec Ge(Li) planar detector (8 mm dia. x 5 mm depth of active volume) cooled with liquid nitrogen is being used in this study. The response of the detector system as a function of photon energies was determined using monoenergetic photons from the emissions of radioisotopes (Fig. 1). The energy response of the detector is linear below 125 keV (the energy of interest in this study). Corrections for detector efficiency and energy resolution were simplified by fabricating experimental parameters so that the detector can be regarded as a point source detector with a special collimator system and an appropriate analyzer adjustment. Figure 2 is a calibration spectrum from the emissions of ^{57}Co at 14.4 and 122 keV. Since each photo peak comprises only three channels and their relative heights are highly reproducible with a given number of photons in the peak channel, the correction for energy resolution can simply be made by adding the number of photons in the two other channels to the main photo peak channel. The efficiency of the detector at 122 keV was determined from Figure 2 by calculating the ratio of the total number of photons under the two photo peaks and comparing it with their relative frequency of emission. The experimentally determined efficiency is 70% as compared to a theoretical value of 67% obtained by using the absorption data of Allen (1) at 122 keV, assuming point source absorption. Correction for the germanium escape peak can be disregarded during spectral correction, and the small numbers of omitted channels in the 10-11 keV regions of the spectrum can be arbitrarily added to the corrected spectrum assuming a smooth function for the true spectrum through that energy interval.[1]

Dosimeter Response

A tandem of two different TLD dosimeters ($CaSO_4$:Dy and LiF) having widely different energy responses are being used in this study to uniquely determine the quality of an x-ray spectrum. Figure 3 shows their energy responses relative to ^{137}Cs. The "effective energy" of an x-ray spectrum is calculated from the first half value thickness (HVT) measurement with the effective energy being chosen arbitrarily to be around half of the kVp.

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[1] A malfunction of the detector in November required manufacturer's service at the factory. This study will resume when the detector is back in service.

We are attempting to study the energy responses of the two TLD dosimeters for a given HVT but with various kVp and added filtration combinations. Also, we are attempting to qualify the term "effective energy" of an x-ray spectrum in terms of the ratio of energy responses of $CaSO_4$:Dy and LiF and depth dose function in water.

The tandem of $CaSO_4$:Dy and LiF dosimeter system will not only provide a unique specification of a radiation spectrum, it will also provide an accurate integral exposure measurement from any unknown x or γ radiation source within the range of 10^{-4} R.[1]

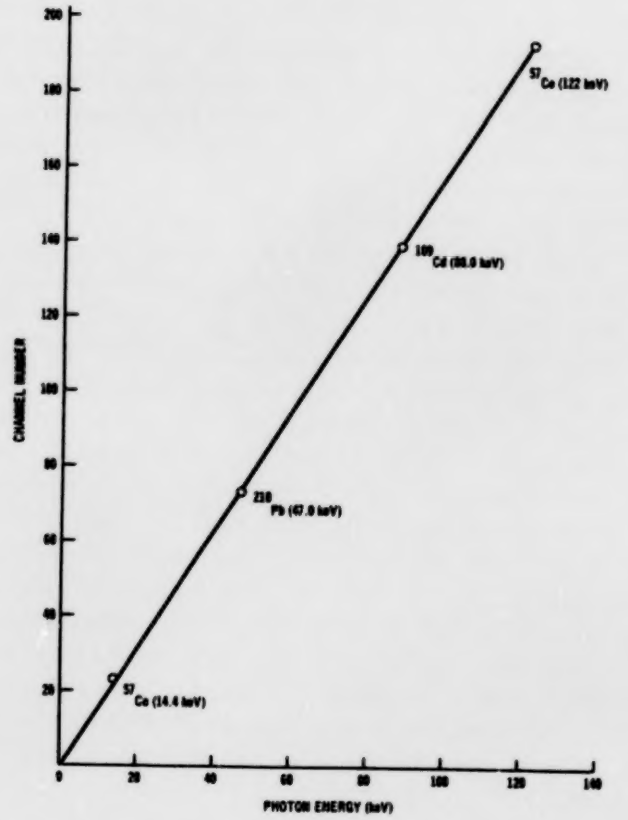

Figure 1. Detector Response as a Function of Photon Energies. Monoenergetic photons from the emissions of radioisotopes were used for the calibration of a Victoreen SCIPP 1600 channel analyzer.

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[1] This estimation is based on the response per roentgen of $CaSO_4$:Dy at 100 kVcp, 9 mm Al HVT to that of LiF. The sensitivity of $CaSO_4$:Dy surpasses that of LiF (TLD-100) by a factor of 146.

If the conventional specifications of an x-ray beam can be correlated to the photon energy spectrum, it will enable the computer to be used for dose calculation to any degree of desired accuracy in cases where physical measurement is inaccessible or impractical.

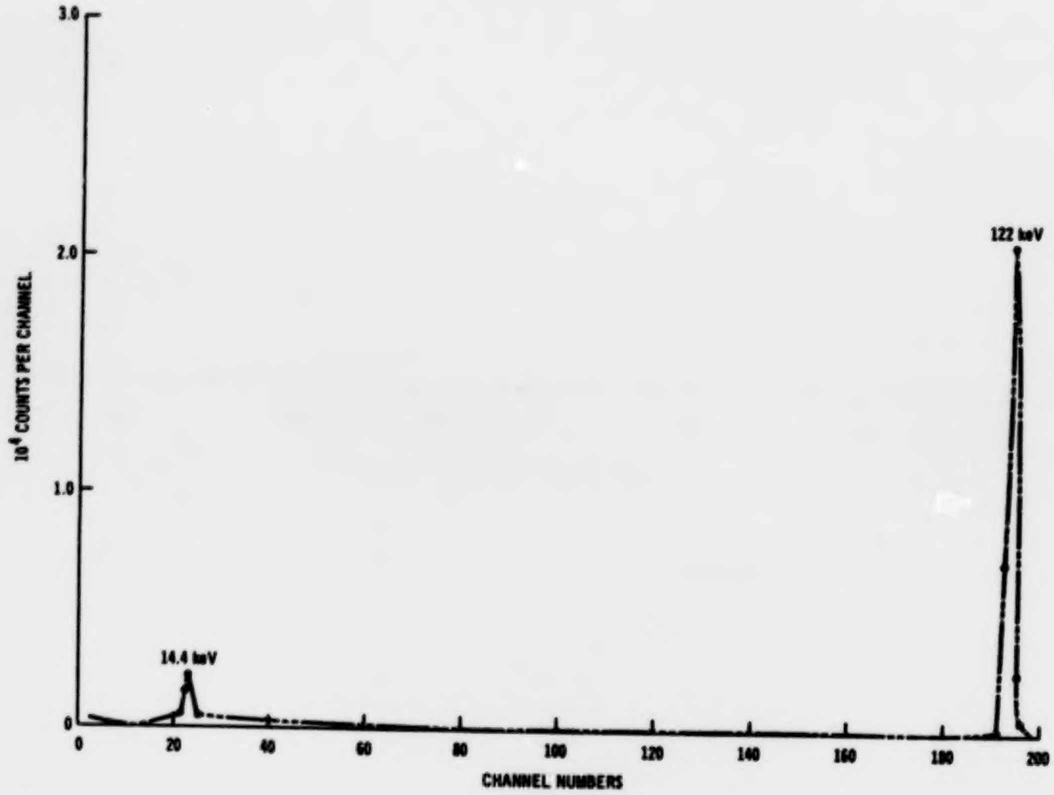


Figure 2. A Calibration Spectrum from the Emission of Cobalt. The total relative counts from the 122 keV spectrum to those of the 14.4 keV one were used for the determination of detector efficiency at the 122 keV region, assuming 100 percent efficiency at 14.4 keV. The Compton peak of the 122 keV spectrum is practically background level.

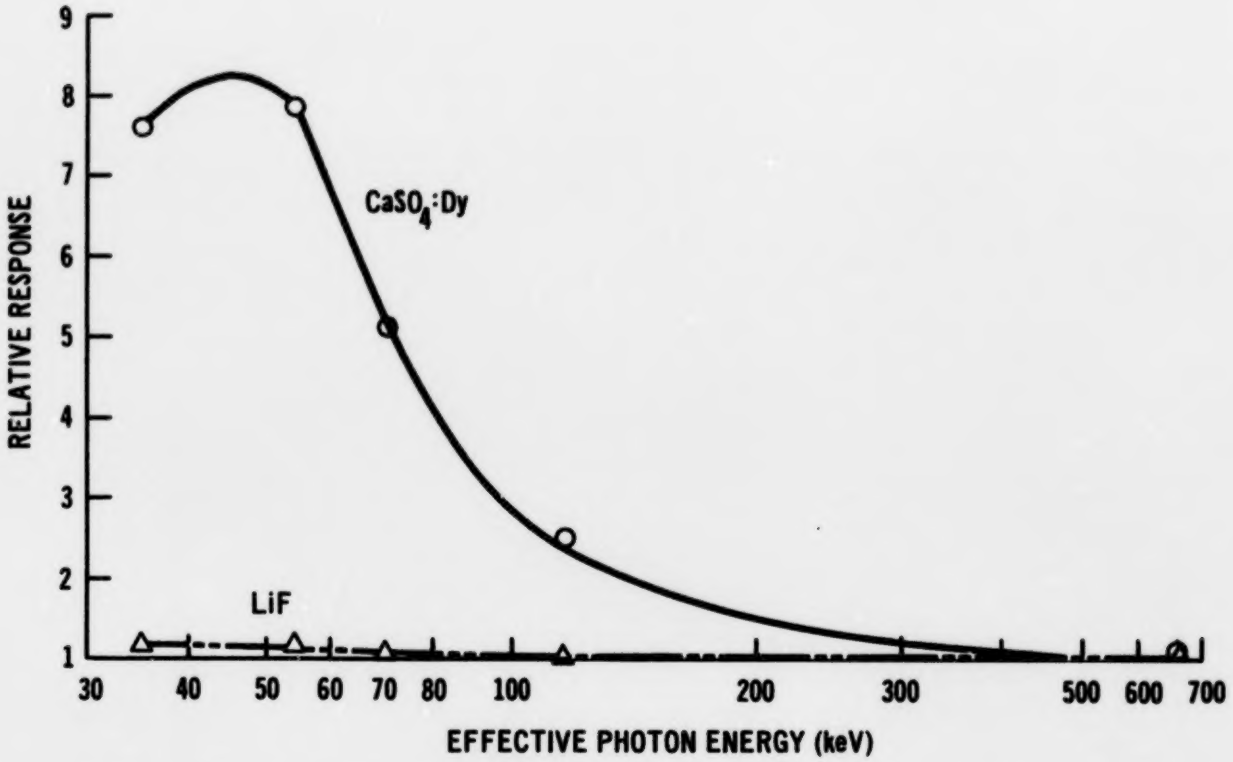


Figure 3. Relative Energy Responses of $CaSO_4$:Dy and LiF (TLD-100) Powders Normalized to 662 keV (^{137}Cs).

Effect of Impurities in Commercial Grade Aluminum Alloys on X-Ray Attenuation.

The energies of photons emitted from electronic products are, in many cases, less than 30 keV. Since an x-ray spectrum is ordinarily specified by its attenuation through aluminum, information on the effect of impurities in commercial aluminum alloys commonly available for x-ray attenuation measurement was needed. Jones (2) strongly recommended that the purity of aluminum be at least 99.8 percent for HVT determination. Unfortunately, most commonly available commercial grade aluminum is about 99 percent pure. Table 1 lists the impurities and HVT's of three commercial grades of aluminum. The experimental HVT's were measured using narrow beam attenuation with an x-ray beam of 30 kVcp, 3.1 mm Al added filtration, 1.1 mm Al HVT. The theoretical HVT values were calculated from the relative mass attenuation coefficients of aluminum alloys to pure aluminum (99.99 + %) at 22 keV effective energy. The impurities in the aluminum appear to yield a maximum effect for HVT measurement at 22 keV effective photon energy (Figure 4).

TABLE 1. CHEMICAL COMPOSITIONS
AND HVT MEASUREMENTS
OF THREE COMMERCIAL-GRADE ALUMINUM ALLOYS

	Composition, %[a]				HVT, mm Al	
Alloy	Al	Fe	Cu	Si	Exp.	Calc.
#3003	97.0	2.0	0.5	0.5	1.05	1.02
#1100	99.0	0.5	0.3	0.2	1.12	1.13
#1050	99.5	0.3	0.1	0.1	1.17	1.16
Pure Al	99.99	-	-	-	1.20	1.19

a. Percentages are based on the chemical composition limits from the Alcoa Aluminum Handbook.

The results of this study indicate that for practical purpose, commercial-grade aluminum alloy of series #1100 or better is adequate for HVT measurements. Since the primary physical characteristic of an x-ray beam is its spectral distribution, which governs its penetration and dosimeter response, it is doubtful that a beam with an HVT of 1.2 or 1.1 mm Al at the same kVp would yield any appreciable difference in penetration and response of any practical dosimeter now in existence. In the future, we will attempt to compare the transmitted x-ray spectra through various commercial grades of aluminum alloys and pure aluminum in order to reaffirm the present conclusion based on attenuation measurements.

References

1. Allen, S.J.M. 1957. Mean values of the mass absorption coefficients of the elements, p. 799-806. In Arthur H. Compton and Samuel K. Allison, X-Rays in Theory and Experiment, 2nd ed. D. Van Nostrand Co., Inc., Princeton, N.J.

2. Jones, D.E.A. 1961. The suitability of materials used for the measurement of the half-value thickness for x-ray beams. Brit. J. Radiol. 34:801-806.

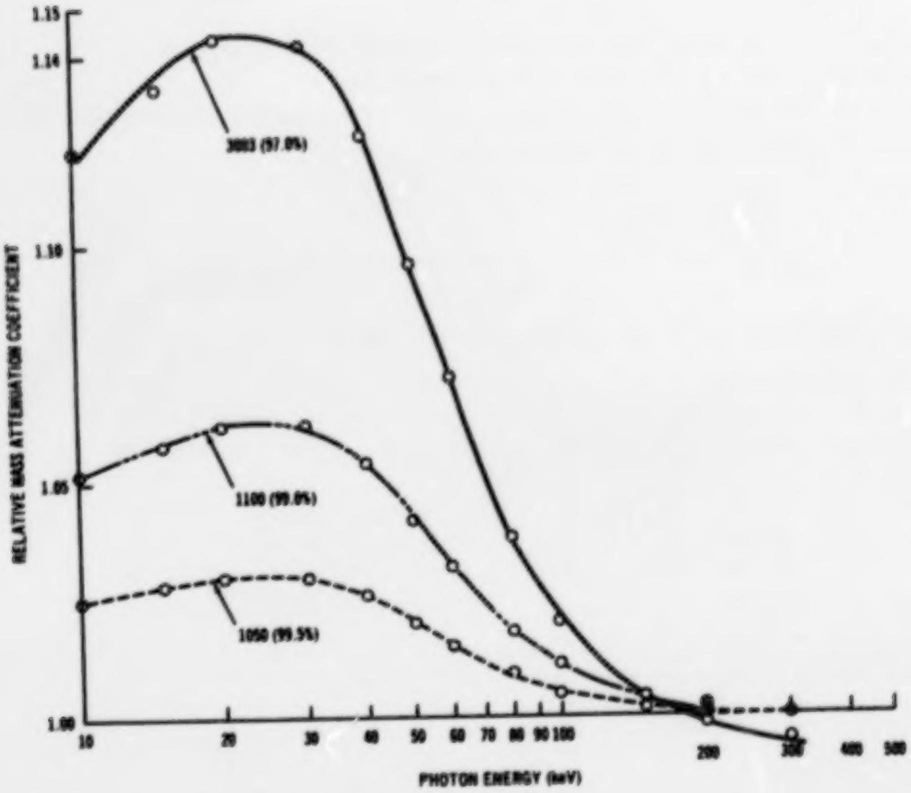


Figure 4. Relative Mass Attenuation Coefficients of Three Commercial Grades of Aluminum Alloys. A 22 keV effective energy beam generated from a 30 kVcp x-ray spectrum with 3.1 mm Al added filtration was used to test the three calculated functions at the maximum effect region with HVT measurements (Table 1).

C. ELECTRONIC INSTRUMENTATION

Temperature Controller Design for a Digital Glow Curve Analyzer

Joseph S. Ali

The design of a special thermoluminescent dosimeter (TLD) reader was initiated in October 1969 to explore the possibility of using TLD powder to measure microwave exposure (1). Design specifications and a block diagram of the temperature controller are included in the referenced article as well as a description of the approach to be taken in the development of the near field probe. Construction and calibration of the temperature controller were completed in July 1970. Shown in Figure 1 are the temperature controller on the left, the Victoreen Scipp multichannel analyzer on the right and a Houston Instruments, Inc. digital plotter with a sample glow curve plot. The high voltage power supply to power the photomultiplier tube in the temperature controller is not shown.

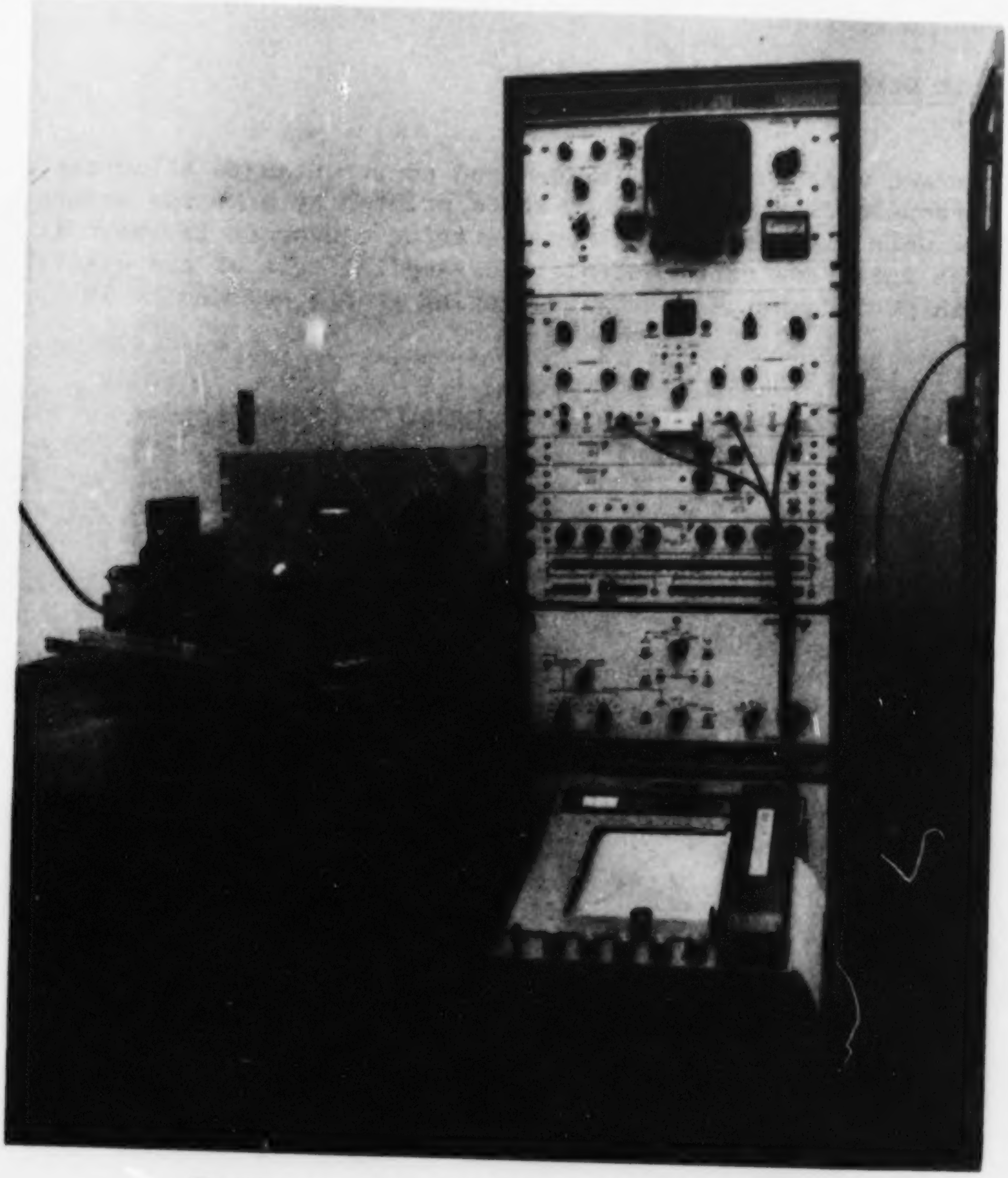


Figure 1. Digital Glow Curve Analyzer.

Phase two of the project, development of the probe, has been postponed due to time commitments on other projects which are described in this report.

Other than being a critical part of the development of a near field microwave probe, the glow curve analyzer can be used to develop better probes for ionizing radiation. Almost all commercial TLD readers display only the integrated light output over a temperature range. By examining the glow curve directly, effects on TLD of various parameters such as annealing procedure, fading of low temperature peaks, heating rate, and mesh size can be studied.

Interface Unit Design

Joseph S. Ali

An interface unit has been designed and constructed to allow the display of a Fairchild digital voltmeter to be printed by a Monroe printer. The interface unit with its associated operating equipment is shown in Figure 2. The interface unit controls the sampling rate of the digital voltmeter with periods between samples ranging from 6 seconds to 14 minutes.

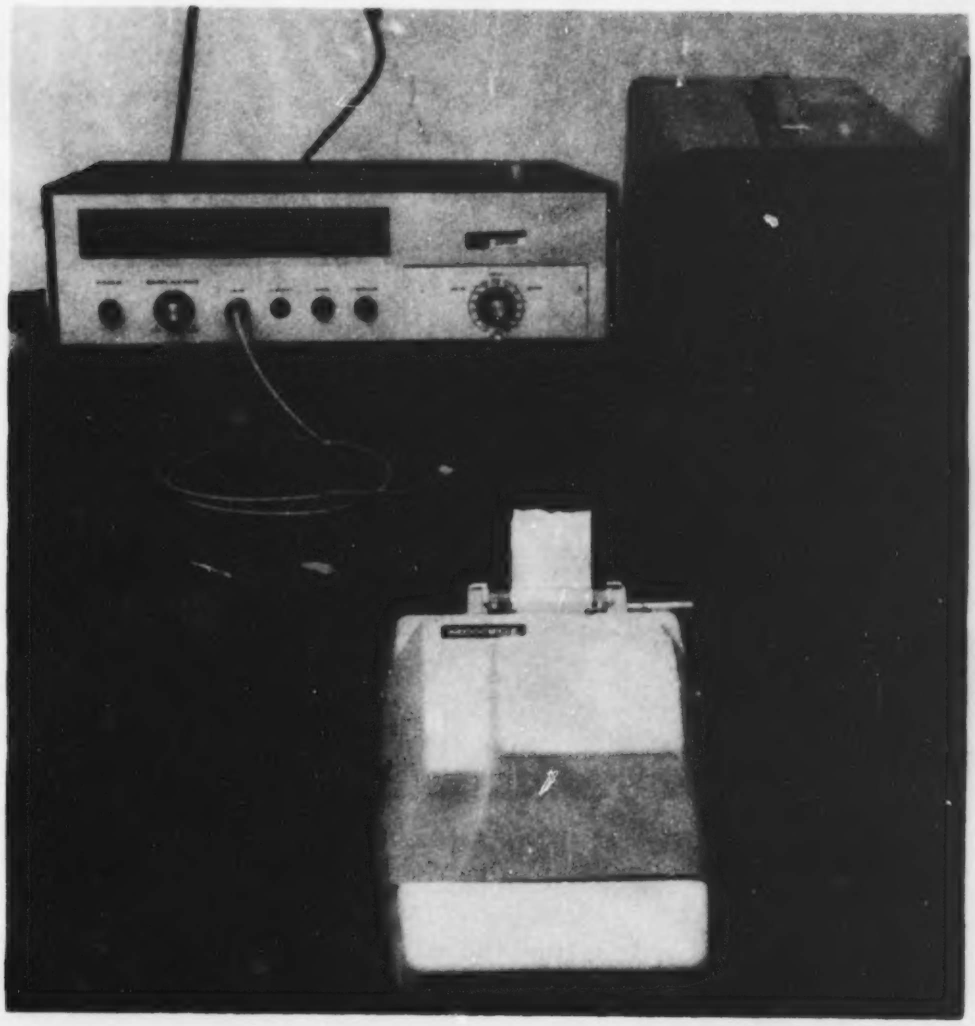


Figure 2. Interface Unit and Associated Instruments.

The unit facilitates gain stability measurements of amplifiers and voltage references. It will be used to evaluate the stability of the nuclear counting equipment in the Toxicologic Studies Section and as a laboratory tool in developing stable circuitry.

Microwave Power Leveler

Joseph S. Ali

The Dosimetric Studies Section provides a microwave exposure facility for the division with a Varian 3,000 watt microwave generator. Since this generator is unregulated, line voltage fluctuations cause the microwave power output to fluctuate. A servomechanism is being designed to control the microwave power output in the presence of line voltage fluctuations. The power leveler will also correct for other disturbances which cause the power output to vary. In addition, the servomechanism will be made compatible with the data acquisition system presently being installed, thus allowing the microwave power to be computer adjustable.

At present, the servosystem design equations have been formulated and an analysis is under way to determine design parameters.

Reference

1. Ali, J.S. 1969. Temperature controller design for a digital glow curve analyzer, p. 159-161. In Radiation Bio-Effects Summary Report, January - December 1969 (BRH/DBE 70-1). U.S. Department of Health, Education, and Welfare, Public Health Service, Bureau of Radiological Health, Rockville, Maryland 20852.

Controlled Environment Facility

George Samaras, George Anderson, and James Rolofson

The Dosimetric Studies Section has become deeply involved in designing and developing experimental exposure facilities to study environmental factors associated with nonionizing radiation, particularly the possible biological implications of microwave exposure. Last year a large anechoic chamber and a new microwave power source were installed at our laboratory to permit investigators to irradiate specimens under more precisely defined conditions than before. A controlled environment system and associated data acquisition system were also planned for use in conjunction with the enlarged microwave exposure chamber.

Technical specifications for the system incorporated such design features and capabilities as follows:

(1) Regulation of the biological test subject's environment through the use of a digital controller. The digital controller maintains the environmental temperature, humidity, air velocity, atmospheric gas concentration, and total pressure. The control of such physical parameters should aid in solving thermal buildup problems which at present are typically encountered during microwave exposures.

(2) Allowance for the investigator to perform multiparameter acquisition of selected environmental and physiological data. Examples of physiological data which can be recorded include electrocardiogram, body and rectal temperature, respiration rate, and expired CO_2 concentration.

(3) Stabilization of selected physiological parameters. For example, by monitoring the test subject's body temperature response and respiration rate response and utilizing a feedback technique with the digital controller, the test chamber environment may be altered as necessary or maintained depending on the final objective of the experiment.

(4) Regulation of the RF source forward power.

Figure 3 identifies the individual components of the entire system which is scheduled to become fully operational during the next calendar year. The alpha-numeric display terminal along with the remote video monitor have been identified as options which may or may not be included.

The biological specimen will be placed in a double-wall spherical chamber constructed entirely of lucite with an interior diameter of 35 inches. The double-wall chamber is used to minimize heat loss and to prevent condensation from forming on the inner or outer surfaces during any chosen combination of exposure conditions. The chamber is mounted atop a moveable car steered along a fixed track system allowing the chamber to be positioned in the near field or the far field of the radiating microwave antenna. The interior of the chamber is accessible by removing the front half of the sphere or by a secondary access port 10 inches in diameter located on the rear half of the sphere. The chamber is connected to the environment temperature control equipment by two 6-inch inside diameter flexible hoses, one an inlet hose and the other a return hose, for routing the pre-conditioned air. The environment temperature control equipment and the microwave generator are a closed loop system, controlled by a small digital computer along with associated peripheral equipment. The facility will provide investigators with a means for achieving controlled biological experiments during microwave irradiation while simultaneously performing rapid data acquisition which can later be subjected to rigorous statistical analysis.

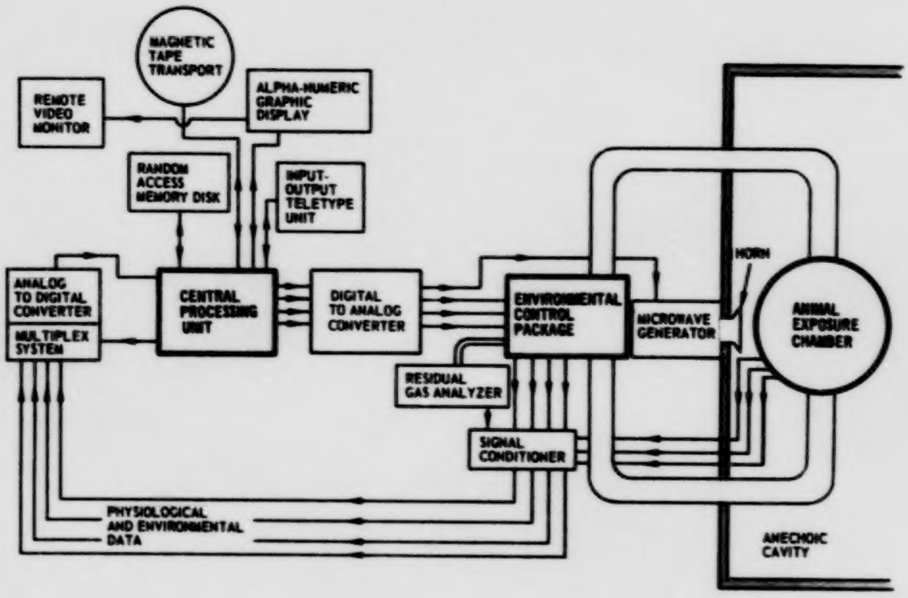

MAGNETIC TAPE TRANSPORT
REMOTE VIDEO MONITOR
ALPHA-NUMERIC GRAPHIC DISPLAY
RANDOM ACCESS MEMORY DISK
INPUT-OUTPUT TELETYPE UNIT
HORN
ANALOG TO DIGITAL CONVERTER
MULTIPLEX SYSTEM
CENTRAL PROCESSING UNIT
DIGITAL TO ANALOG CONVERTER
ENVIRONMENTAL CONTROL PACKAGE
MICROWAVE GENERATOR
ANIMAL EXPOSURE CHAMBER
RESIDUAL GAS ANALYZER
SIGNAL CONDITIONER
PHYSIOLOGICAL AND ENVIRONMENTAL DATA
ANECHOIC CAVITY

Figure 3. Controlled Environmental System.

During 1970, the system progressed from the most elementary stages of planning and design, through completion of the written specifications for the necessary equipment, to the procurement of many of the individual components.

To date the data acquisition equipment, residual gas analyzer, operator's console, and the environmental conditioning and control equipment have arrived. Figure 4 shows a view of the control console for the facility along with some of the associated components. Water and power connections in the near future along with expected delivery of the chamber before the year's end, will allow personnel of the Dosimetric Studies Section to begin a considerable period of testing and evaluation.

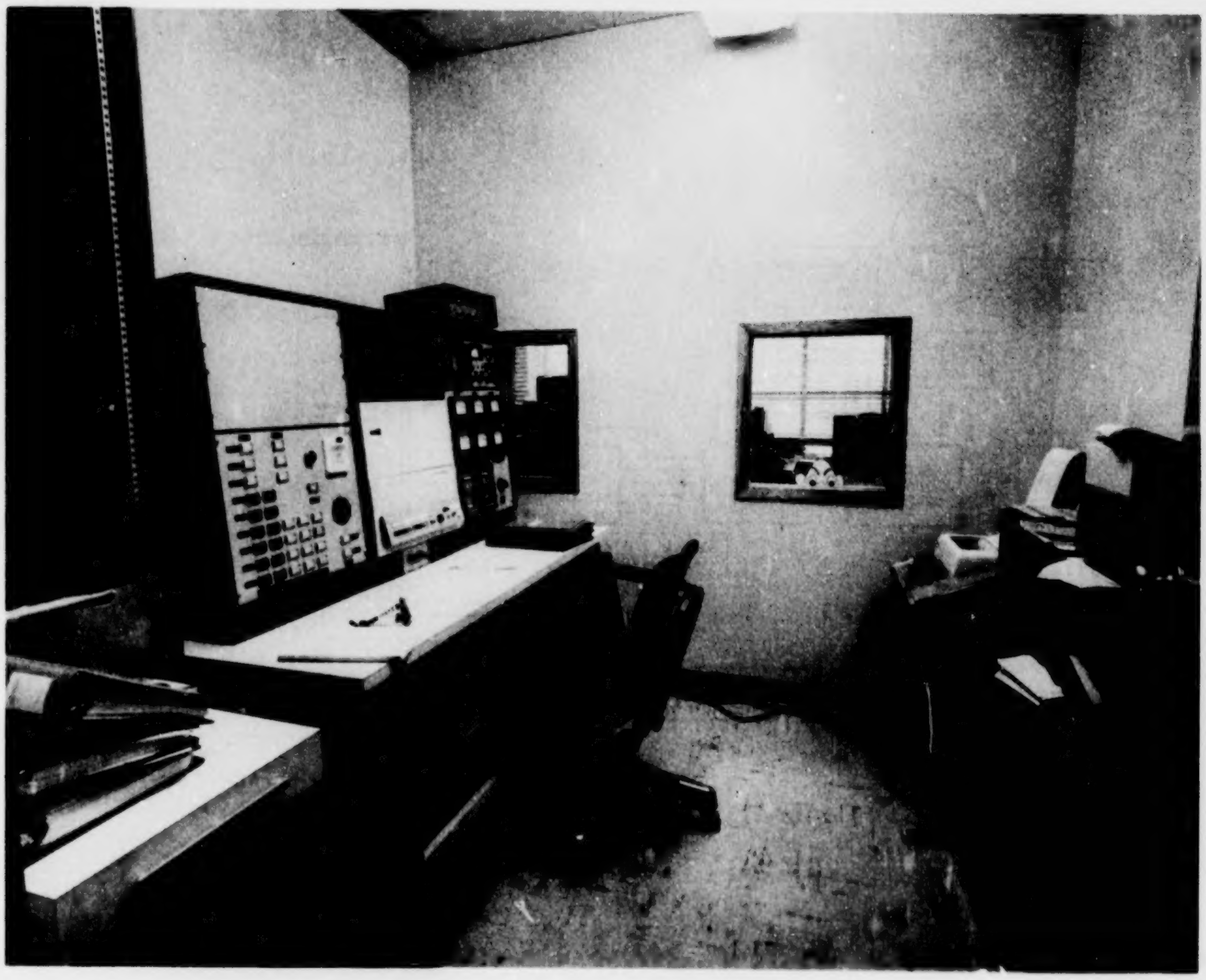


Figure 4. View of the Control Console of the Controlled Environmental Microwave Facility.

D. MICROWAVE DOSIMETRY TECHNIQUES

Radio Frequency and Microwave Energy Absorption in Tissue

R. A. Tell

The problem of microwave dosimetry in humans and laboratory animals is complicated by the critical relationship of power absorption upon tissue parameters. Unlike high energy photons, such as x rays, microwaves may be strongly reflected by an interface between two different tissues. This can mean that the total energy, or power, extracted from an incident beam of microwaves may vary from a small amount to essentially 100 percent.

An analysis of this situation as applied to a plane slab model, as depicted in Figure 1, may be made utilizing transmission line calculation techniques. Using the complex relative permitivities and thicknesses of the materials, the surface impedance of the model may be computed and from that result the percentage of total power absorbed by the system can be obtained. It is assumed in this model that the base layer of muscle is semi-infinite in extent; i.e., there are no underlying reflective surfaces to which a wave might penetrate. The depth of penetration for muscle at this frequency, that thickness which attenuates the wave by 1/e or to approximately 37 percent of its initial intensity, is about 1.98 cm.

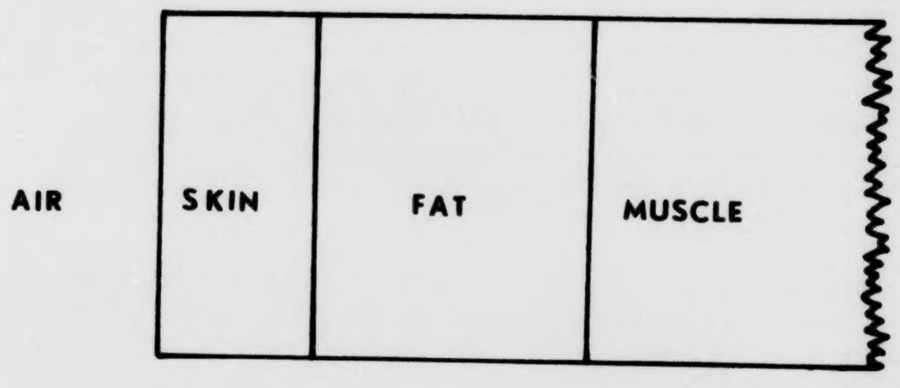


Figure 1. A Section of Plane Slab Model of Tissue System.

The general form of the impedance at any point p through the tissue system is given by

$$Z_p = Z_o \frac{Z_1 \cos h\, \gamma d + Z_o \sin h\, \gamma d}{Z_o \cos h\, \gamma d + Z_1 \sin h\, \gamma d}$$

where Z_o refers to the complex characteristic or intrinsic impedance of the material containing the point p, and Z_1 is the complex effective load impedance for the propagating wave. γ is the complex form of the propagation constant where $\gamma = \alpha + j\beta$, α being the attenuation factor and β the phase factor. d is the distance of point p from the interface defining Z_1. Γ, the voltage reflection coefficient at the air-tissue interface is related to the surface impedance of the model by

$$\Gamma = \frac{Z(\text{surface}) - Z(\text{air})}{Z(\text{surface}) + Z(\text{air})}$$

From Γ, the amount of power absorbed by the system, $1 - |\Gamma|^2$, can be computed.

Figure 2 illustrates the results of these computations of incident power which is absorbed by the system as a function of fat thickness for several different skin thicknesses at a frequency of 2450 MHz, Notice the influence of the fat layer on absorption of power by the system. The maximum absorption peaks are observed to have a spacing equal to one-half of the wavelength in the fat; it can be interpreted that the fat layer acts as an impedance transformer with optimum coupling occurring at fat thicknesses equal to $\lambda/2$ beyond the first peak. Also, beyond 4 mm of additional skin tissue there is little change in the absorption picture. Values for the complex relative permitivities were taken from the literature (1) and are considered to be representative of tissues which would be found in humans ($\epsilon^*_{skin} = 42.9 - j14.0$, $\epsilon^*_{fat} = 5.83 - j1.01$, $\epsilon^*_{muscle} = 47.6 - j13.7$).

These calculations are being extended to include other frequencies and to determine the heat distribution, i.e., where the thermal dose is delivered throughout the system. Using information on the thermal properties of the tissues, estimates will then be made to ascertain approximate temperature rises which could be expected at various points in the model system. Comparison of these theoretical predictions will be made with experimental measurements of temperature distributions in dielectrically equivalent models. This work represents a first approximation to the much more difficult problem of analytically specifying the heating in an irregularly shaped model.

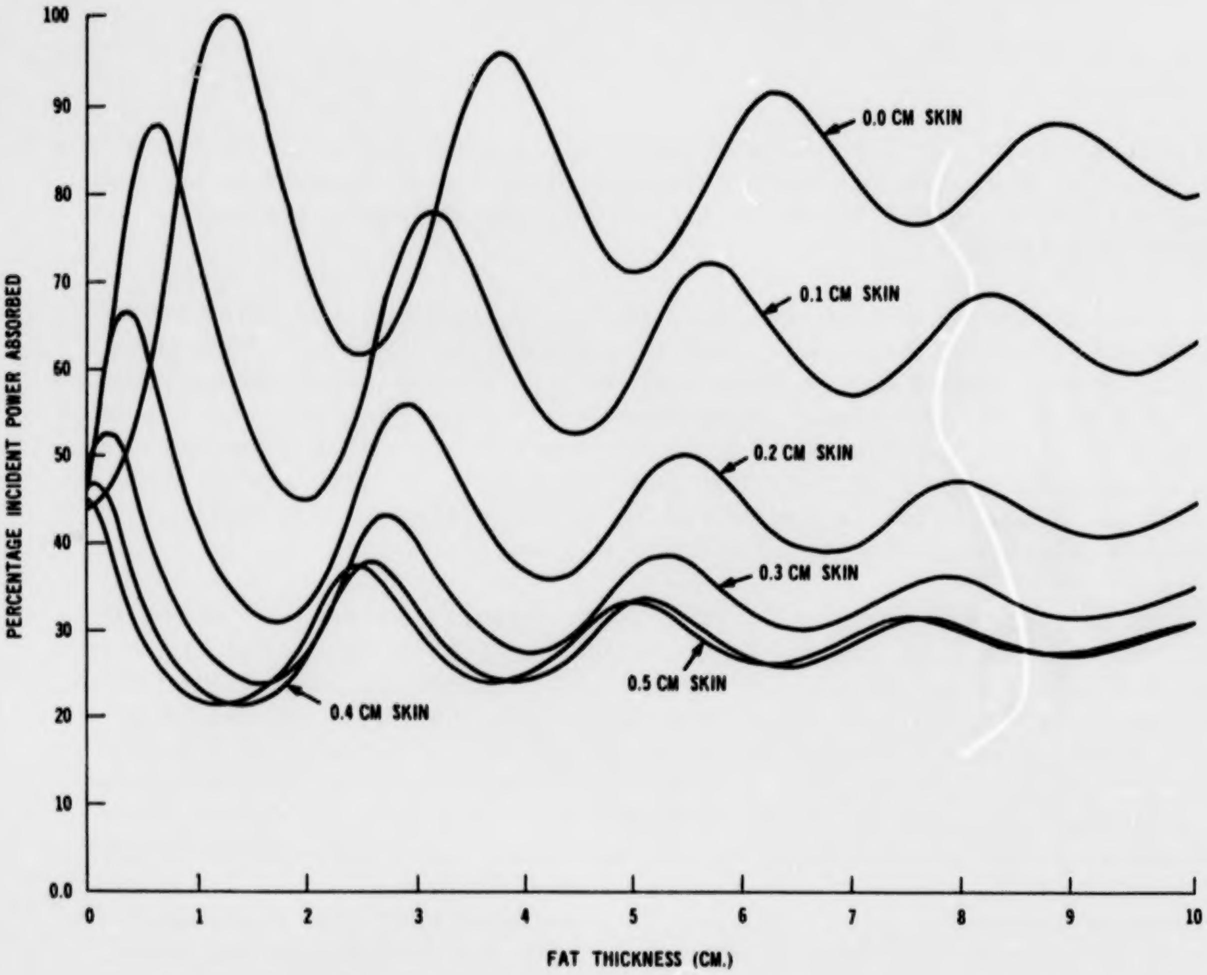


Figure 2. 2450 MHz Microwave Power Absorption in Tissue.

Reference

1. Schwan, H.P. 1958. Biophysics of diathermy, p. 55-115. In Therapeutic Heat. Elizabeth Licht, Publisher, New Haven Conn.

Heating With Diathermy

R. A. Tell, J. B. Kinn

In cooperation with the diathermy study in the Epidemiologic Studies Branch (see p. 50), the Dosimetric Studies Section is investigating the thermal dose which might be delivered to the eye during a typical diathermy treatment.

A loan of the diathermy machine, Figure 3, used in the treatments was arranged with the National Institute of Dental Research, Bethesda, Md., and several measurements have been made. Of these preliminary estimates, one will be described. More detailed information will be obtained from work which is continuing in our laboratory. The diathermy machine operates at a frequency of 2450 MHz and is capable of developing about 125 watts of power which is delivered to the applicator, the device which directs the microwave energy toward the patient.

Material, dielectrically equivalent to muscle tissue, was modeled roughly to the shape of the human eyes and fixed in a de-fatted, commercially available human skull, the interior of which was also filled with the muscle-like material. The artificial tissue was also placed over the jaw area and other facial parts to simulate, as closely as possible, the actual case. Thermocouple probes were used to monitor the midpoint temperature of each eye and a measurement was also made of the central temperature of the skull interior. Measurements were made continuously for a period of 30 minutes, and are indicated in Figure 4 for a typical treatment of the right jaw. Usually, the patient held the applicator in position just below the lobe of the ear, directed toward the jaw area, with a spacing of about 1 inch from the applicator to the skin surface.

The data suggest that heating of the right eye was nearly linear over the period of treatment with a temperature rise of about 0.2°C per minute while the heating of the left eye appears unnoticeable. A slight rise in central skull temperature is evident toward the termination of the irradiation. Though these measurements are only very approximate in nature, they are probably not drastically different from the actual thermal burden in the live person. Because of the placement of the absorptive muscle material on and throughout the skull, we believe our measurements of resulting temperatures are not unrealistically high. Thus, for treatments as long as 8 minutes, the maximum time for this specific technique, it appears that an elevation of 1.6 C might occur at the midpoint of the eye on the treated side.

More refined experiments will be conducted to determine temperature rises very near the surface of the eye.

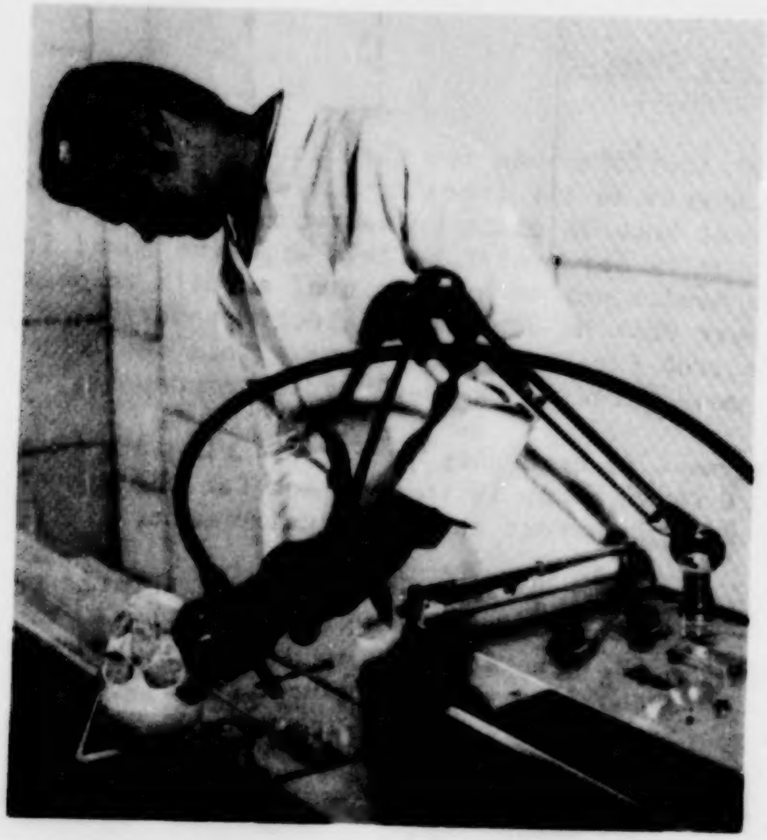


Figure 3. Diathermy Machine with Applicator.

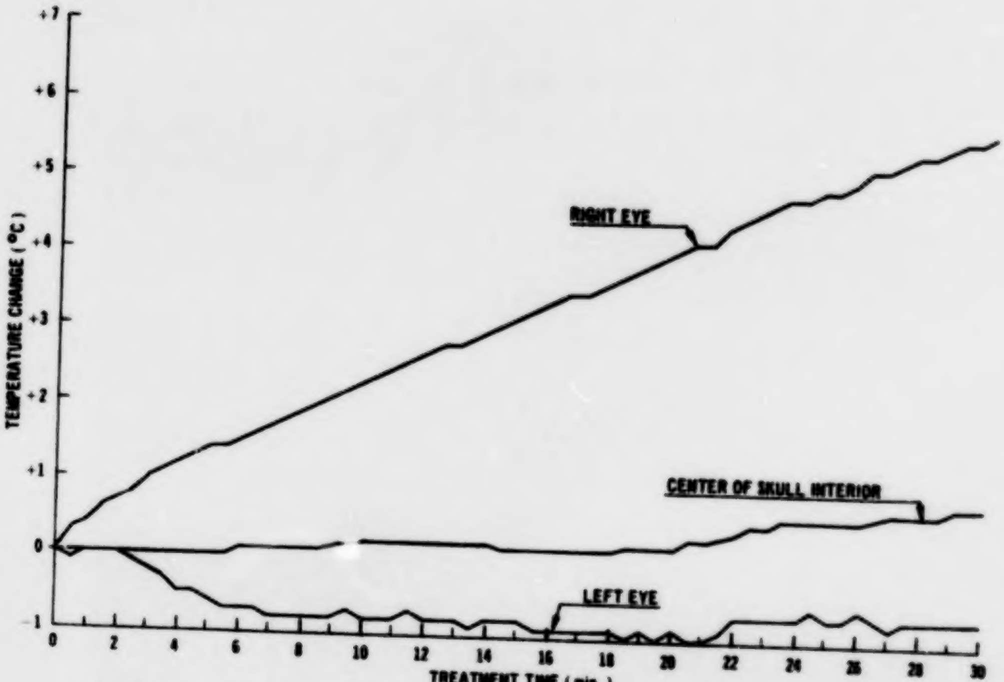


Figure 4. Eye Temperature Vs. Irradiation Time in Diathermy Treatment of Right Jaw.

Evaluation of DBE Anechoic Chamber

D. L. Dawes and G. E. Anderson

At present the most flexible mode for the microwave irradiation of a large variety of targets is the free far-field configuration. Although the greatest real hazards exist in near-field situations, present standards are written in terms of far-field exposure conditions; therefore, for exposure conditions of experimental animals to be related to standards, the exposure must be done in an electromagnetic environment as close as possible to true far-field conditions. This excludes cavity-type exposures and for many targets, intra-waveguide exposures.

An anechoic environment is necessary to closely simulate far-field exposures. An essential requirement is to know how far the actual exposure conditions depart from a perfect anechoic environment.

The anechoic chamber in the Division of Biological Effects (Figure 5) was evaluated by comparing antenna patterns as described by E. F. Buckley, Microwave Journal, August 1963. The quantity measured is the ratio of the vector sum of all reflected and diffracted components of the vertically polarized electric vector to that of the main transmitted vertically polarized electric vector in the main beam.

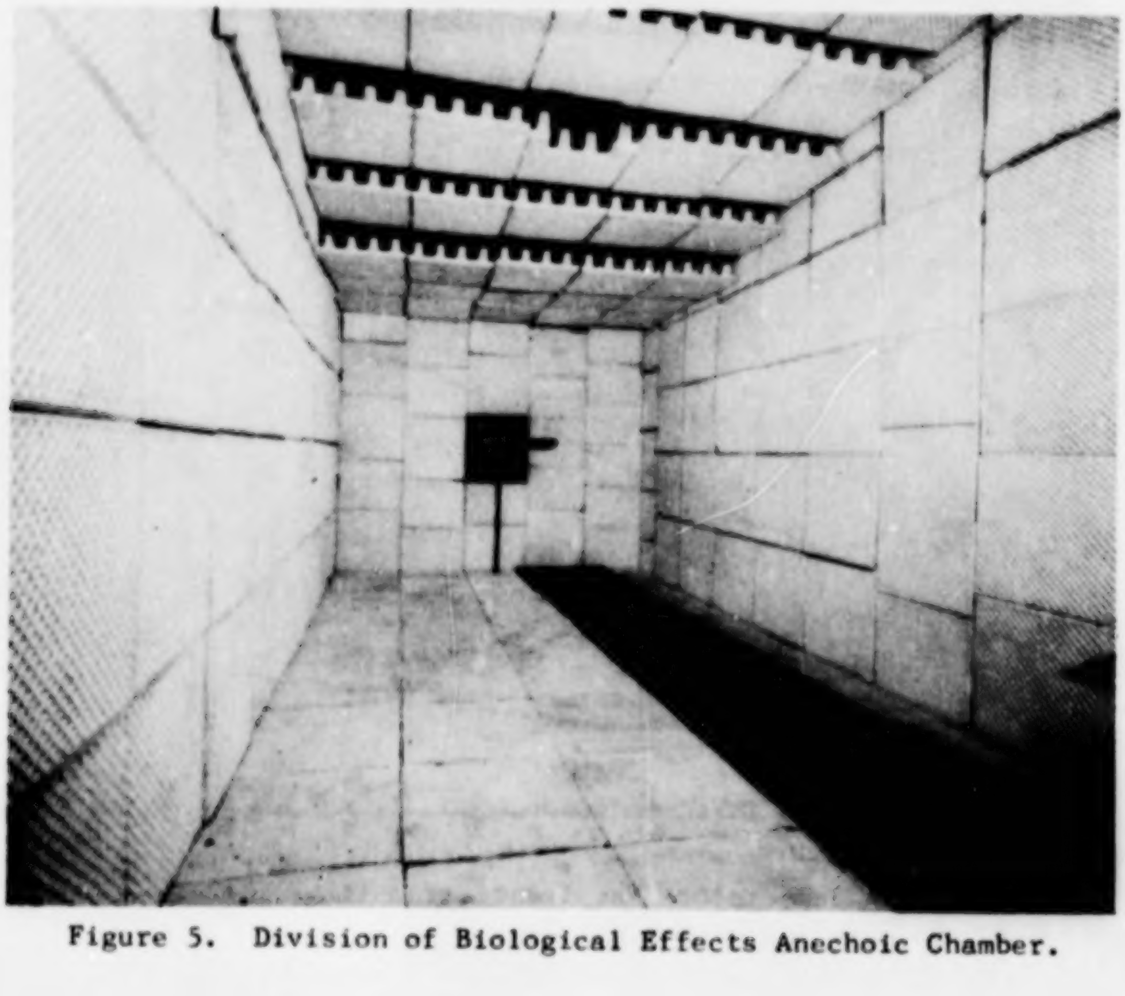


Figure 5. Division of Biological Effects Anechoic Chamber.

The measuring apparatus is pictured in Figure 6, and is an example of standard techniques used to measure low intensity reflections. Figure 7 shows a typical azimuthal scanning pattern.

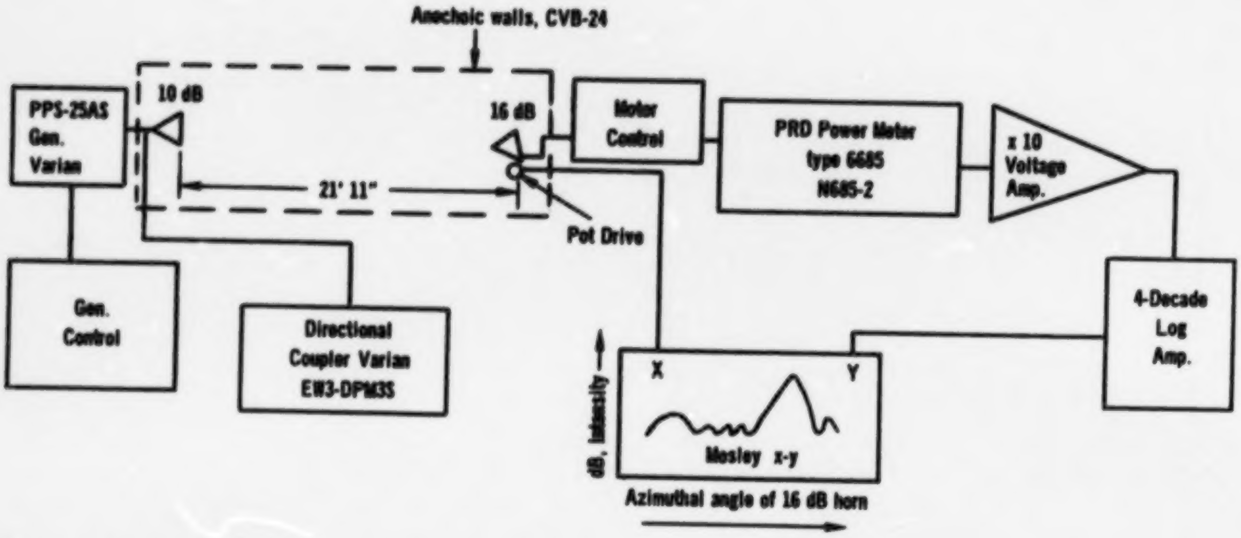


Figure 6. Schematic of the Setup Used to Take Azimuthal Scans to Measure Reflections Off the Surfaces of the Anechoic Chamber. Where the manufacturer is not indicated, equipment was built in-house.

The anechoic chamber measures -36 dB ± 2 dB, i.e., on the average, reflections in the quiet zone were 36 dB less than the intensity of the main beam. This means that, on the average, the power impinging on the target due to reflections and diffractions was 3,980 times smaller than the power available from the main beam. The quiet zone could roughly be described as a truncated cone centered on the beam axis with an approximate 2 ½-foot radius at the rear of the chamber and expanding toward the transmitting horn.

Since the chamber evaluation is dependent upon the horns used, it must be emphasized that this characterization assumes a 10 dB pyramidal transmitting horn and a 16 dB pyramidal receiving horn used to take azimuthal scans.

Although the chamber was computed to have an average reflection ratio of -36 dB, peaks off the main and rear lobes of the receiving horn measured as high as -27.4 dB at points 3 feet from the main axis. The ratio of reflected to incident power at a point will decrease as the point approaches the main axis and the transmitting horn. As a rule of thumb based upon viewing the antenna pattern scans, the average ratio of reflected energies to incident energy averages near -30 dB, which puts the magnitude of the echoes near a factor of 1,000 less than the main beam intensity. This anechoic chamber therefore is adequate for biological exposures and rough antenna pattern mapping.

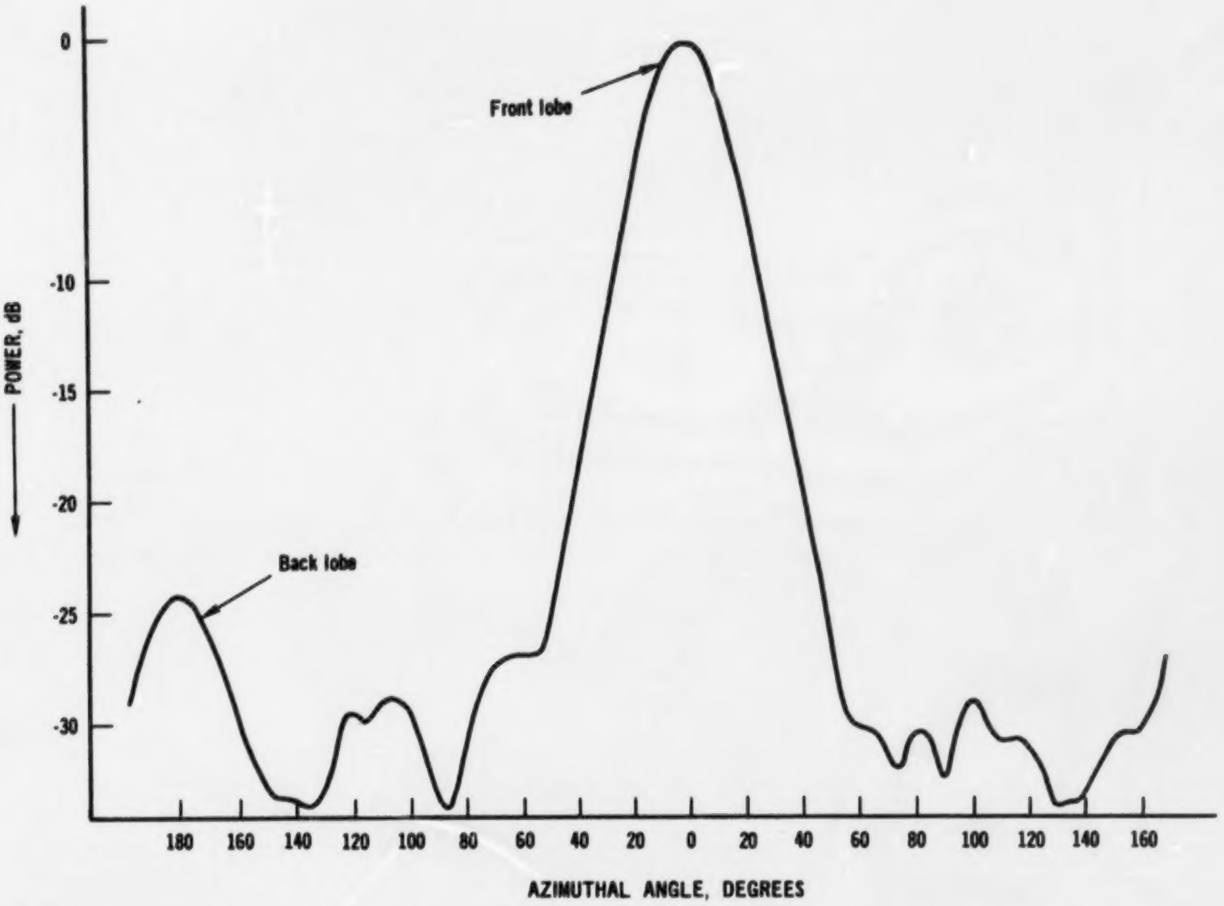


Figure 7. Azimuthal Antenna Scan at 2450 MHz on the Main Beam Axis. Scan made with a 16 dB pyramidal horn at a distance of 21 feet 11 inches from the 10 dB pyramidal transmitting horn.

Measurement of Absorbed Microwave Energy in Biologically Equivalent Phantom Models

J. B. Kinn

Current practice in microwave dosimetry is usually limited to specifying free-field exposure values in terms of power density. Although technical complexities have strongly influenced this convention, the more meaningful measures of absorbed energy, or dose, and dose distribution are possible. The dosimetric techniques discussed here were aimed at defining microwave dose and dose distribution as applied to biologically equivalent models, or phantoms, of various geometries. The approach used in our laboratory, similar to that developed elsewhere (1), involves the construction of a dielectric phantom model, exposure of the model to a microwave field, and observation of the temperature distribution within the model. From the temperature data and the materials' thermal properties the actual energy distribution can be computed.

Liquid crystals were chosen to measure the temperature distribution. These encapsulated crystals are deposited on a mylar sheet which is brought into contact with a flat surface of an exposed model. Heat transferred to the liquid crystals is displayed as a color change on the mylar sheet which is photographed. A metallic bar with a known thermal gradient provides the color-temperature calibration of the liquid crystal sheet.

After calibration, a color comparison technique is used to convert the observed color pattern to the actual temperature distribution.

Presently, several different types of phantoms have been constructed. These include two layer (fat-muscle) plane slab models as well as spherical designs (a solid fat equivalent model and a concentric shell model composed of an exterior shell of fat and a muscle interior). An electrically equivalent human skull and monkey skull are nearing completion and will be used in future work.

Preliminary exposures of the solid fat spherical phantom reveal the development of a definite hot spot, eccentric to the center and away from the incident wave. The heating pattern in a 12.6 cm fat shell filled with muscle showed no evidence of such a hot spot. Figure 8 gives a qualitative, pictorial representation of the heat development distribution in the spherical models. The observed experimental thermal distributions are being compared with theoretical predictions. Additionally, measurements will soon begin to determine the actual complex dielectric constant of the modeling materials.

Reference

1. Guy, A.W., J.F. Lehmann, and J.A. McDougall. 1968. Studies on therapeutic heating by electromagnetic energy. In Thermal Problems in Biotechnology. American Society of Mechanical Engineers, New York, N.Y.

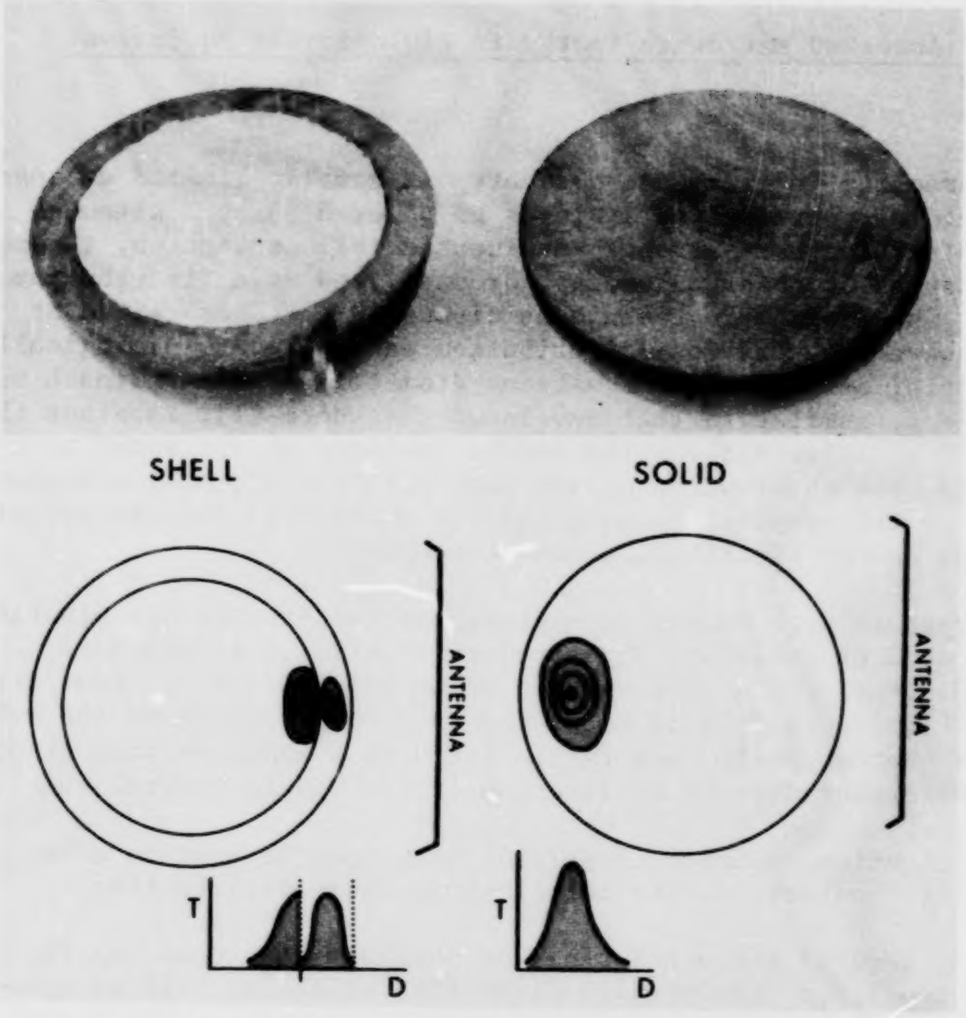


Figure 8. Spherical Phantoms used in Microwave Energy Absorption Studies. Spherical shell phantom is filled with muscle-equivalent material. Drawings indicate location and magnitude of microwave heating in spherical models.

TOXICOLOGIC STUDIES SECTION
Dr. James F. Wright, Chief

In order to assist other Bureau programs to determine the environmental and critical population dose effects of hazardous radionuclides associated with power reactors and nuclear fuel reprocessing plants, the Toxicologic Studies Section is seeking to determine whether there are variations in sensitivity from exposure to radiostrontium during periods of organogenesis, growth, and development using the cat as an experimental animal.

Presently there is no doubt that neoplastic disease of the hematopoietic system rather than the skeletal system is the more important biological effect of exposure to radiostrontium, but the question of the relative sensitivity of various age groups to these exposures is virtually unknown. Since radiation protection standards advocated by such groups as the Federal Radiation Council, International Commission on Radiological Protection, and National Council on Radiation Protection and Measurements are based on the "average man," it must be determined what kind of extrapolations can be made to other populations (fetal, neonatal, adolescent) in the establishment of exposure guidelines.

Several alternatives have been considered in developing the basic experimental design for this study; these are based on the limitations imposed by the colony capacity and the need for statistically significant numbers of animals in each experimental group. A final design that includes four age groups and three dose levels of radiostrontium seems most likely to offer the most information under these conditions. Doses will be related, at the lowest level, to current maximum permissible body burdens of the isotope.

During the past year two age groups of cats were exposed to three dose levels of strontium 89 in a pilot study designed to add to information on the body burden accretion rates for various age groups and to accurately define dose-response relationships. This study and other ancillary metabolic and dosimetric studies are described in the sections that follow.

Two of the three colony facility improvements necessary for the experimental work have been recently completed. The colony support building was modified to meet radiation safety requirements, and a whole-body counter was fabricated and installed in this area. The third facility improvement, for which funds have not been available, is the additional animal run originally planned for the colony.

A. STRONTIUM-89 PILOT STUDY

N. Nelson, E. Berman, J. Wright, J. Ward, and S. Wilson

Two ages of cats, weanling and adolescent, were selected for this study, which was designed to answer questions relating to the strontium 89 "core" age sensitivity study. We expected a number of parameters to vary due to the age-dose relationship in this study; of these parameters, blood-formed elements and growth by weight were expected to be influenced most. Because of this, blood sample collections and weighings were prescheduled and every effort was made to adhere to the schedule.

Methods

Four dose levels were used: 100, 50, and 25 μCi/day and sham. Six litters of weanling animals were used with no more than one littermate in each dose level. Littermates could not be obtained for each dose level in the adolescent group, so five adolescent animals were placed in each dose level using littermates where possible. Four of the animals in each dose level were to be maintained for 180 days after dosing. One animal in each dose level was sacrificed on the day after the last dose for dosimetric purposes. A sixth animal in the weanling dose levels was added to insure that four animals would be available at the end of the study. The radiostrontium dose was administered between the hours of 1 and 2 p.m. The different dose levels were administered randomly to minimize any possible effect from the order of dosing. Thirty consecutive daily oral drench dosere were administered.

Weanlings were kept as a group in pens separated from the older animals, up to eight weanlings per pen. Older cats were separated into groups of six. Cats of one dose level were not separated from those of another. At the scheduled date, each cat was fed C/D feed only. No other feed, supplement, or additive was given. Vaccinations and piperazine were given routinely. Illnesses or deaths were reported on the day first observed. The time found dead, position found, location found, estimated time of death, and any pertinent observations were noted. All postmortems were performed at time of sacrifice or as soon after natural death as possible.

Blood samples were collected from the jugular vein into 2.0 ml vacutainers containing EDTA. The older group donated a full vacutainer; the weanlings only 0.5 ml. Collections were made before 11:00 a.m. of the scheduled day and taken without delay to the clinical pathology laboratory. Each sample was inspected for clots before being taken to clinical pathology; clotted samples were repeated the same morning. Animals were weighed periodically before noon of the scheduled day.

Shortly after the start of the dosing, weanlings on the 100 μCi/day dose level showed acute radiation effects. Since the experiment was designed to show 30-day radiation effects and recovery, representative animals had to survive the dose period. To have a better chance of showing recovery from high-dose levels, an additional six weanlings, not littermates, were added to the weanling group and dosed at 75 μCi/day.

Mortality data are shown in Table 1 and Figure 1. Weight was influenced primarily in weanling animals. In the adolescent group of animals, the weights of sham and treated cats increased at about the same rate. However, during the first 30 days, weights in the weanlings increased at a normal rate only in the sham and 25 μCi/day dose groups. Animals in the 50 and 75 μCi/day dose groups stopped gaining weight on about the 11th day after their first dose and started losing weight by the 20th to 25th day. The 100 μCi/day dose group stopped gaining by the 5th day

TABLE 1. MORTALITY IN FELINES
ADMINISTERED ^{89}SR ORALLY, DAILY FOR 30 DAYS

Age Group	Dose Ci/day	Died	
		Percent	Fraction
Weanling	100	100	6/6
Weanling	75	83	5/6
Weanling	50	17	1/6
Weanling	25	0	0/6
Weanling	Sham	0	0/6
Adolescent	All	0	0/5

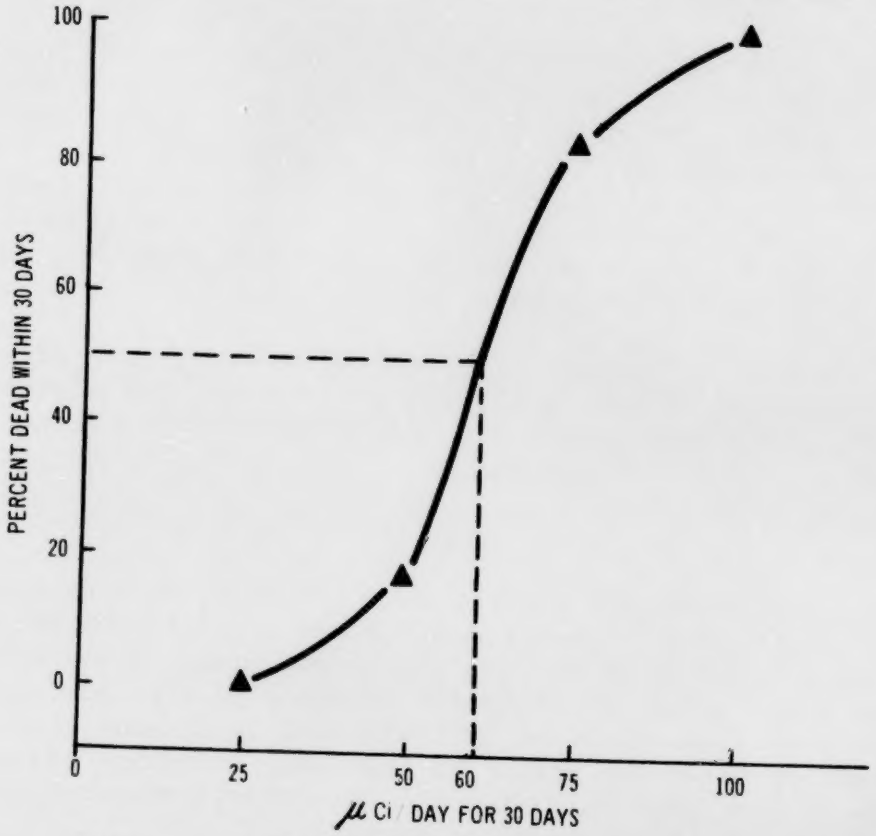


Figure 1. Thirty-Day Mortality in Weanling Kittens Fed Strontium 89 for 30 Days.

and started losing weight by the 15th day. Animals in the 25 μCi/day group stopped gaining on the 30th day and on the 40th day were 10 percent lower in weight than shams. Animals that lost weight continued losing until the day of death or sacrifice.

The blood in both weanling and adolescent groups showed radiation alterations (Table 2). Red blood cells were least affected during the 30-day dosing period; adolescents showed no appreciable change in red cell count, hemoglobin, or packed cell volume, and weanlings showed only a moderate depression of these values. However, weanlings displayed a more pronounced reduction in packed cell volume (30-50 percent) in the 50, 75, and 100 μCi/day dose levels in the last 5 days of dosing and in the red cell counts at 30 days after the start of dosing.

White cell counts were profoundly influenced in the adolescents starting at about the 12th day of dosing. The depression in count at the 30th day was dose dependent going from about 15 percent in the 25 μCi/day group to about 70 percent in the 100 μCi/day group. This depression was caused by a drop in the polymorphonuclear cells. The lymphocytes showed no apparent radiation-related decrease. A similar but much more rapid pronounced effect was noted in the weanlings. Depression in white cell count was evident by the 2nd day after the start of dosing; at the 30th day, depression ranged from about 78 percent in the 25 μCi/day group to about 88 percent in the 100 μCi/day group. Again the depression was caused by the loss of polymorphonuclear cells from the population rather than by loss of lymphocytes.

Platelets also were greatly influenced with depression starting on about day 13 of dosing in the 100 μCi/day weanling group and day 20 in the 25 μCi/day group. The rate of depression was extremely rapid, falling from 100 percent of the sham level to near 0 in 15 days. Effects were less pronounced in the adolescent group, starting later (20-24 days) and not dropping as far (65-20 percent of sham) as in the weanlings. Animals in the 25 and 50 μCi/day groups appeared to recover, but the platelet counts in the 100 μCi/day group remained depressed.

The general picture at 120 days after dosing was that of recovery in the 25 μCi/day weanlings, 25 and 50 μCi/day adolescents, but continued depression of most parameters in the 100 μCi/day adolescent group.

Pathology

Gross pathololgic findings, similar in all animals dead to date and varying only in degree, were those of a radiation-induced hemorrhagic syndrome. In all animals the most prominent gross change was diffuse reddening of the lymph nodes with moderate increase in size generally. Hemorrhages were most often noted in the subcutaneous tissues (Figure 2), myocardium (Figure 3) below the serosa at the small intestine (Figure 4) and less frequently the large intestine. A few animals showed hemorrhages along the cranial suture lines (Figure 5).

TABLE 2. EFFECTS OF STRONTIUM 89 DOSING ON BLOOD CELLS

Age	Dose µCi/day	Start of Depression, day	Percent of Norm 30 days	Percent of Norm 90-120 days
		White Blood Count		
Weanling	25	Immediate with a slight plateau from days 4 to 12	23	140[a]
	50		21	all dead
	75		17	25[b]
	100		12	all dead
Adolescent	25	12	65	88
	50	12	40	65
	100	12	28	22
		Red Blood Count		
Weanling	25	29	92	130[a]
	50	26	70	all dead
	75	21	55	50[b]
	100	13	70	all dead
Adolescent	25	No specific drop noted	100	100
	50		90	100
	100		100	46
		Platelets		
Weanling	25	20	20	100[a]
	50	15	5	all dead
	75	15	1.5	50[b]
	100	13	0	all dead
Adolescent	25	24	65	90
	50	24	40	60
	100	21	20	3

a. Data from two survivors.
b. Data from one survivor.

Free blood or bloody fluid was not present in the body cavities except for one case with a severe fibrinous pleuritis and pneumonia. Hemorrhages in the wall of the urinary bladder occurred in a few cases, but not all of these had evidence of bloody urine. In others the bladder urine was discolored with blood without gross evidence of bladder hemorrhage. Hemorrhage into the intestinal lumen was seldom found but evidence of hemorrhage from the nose and gums was frequently present. In general there was little evidence of marked blood loss from any site.

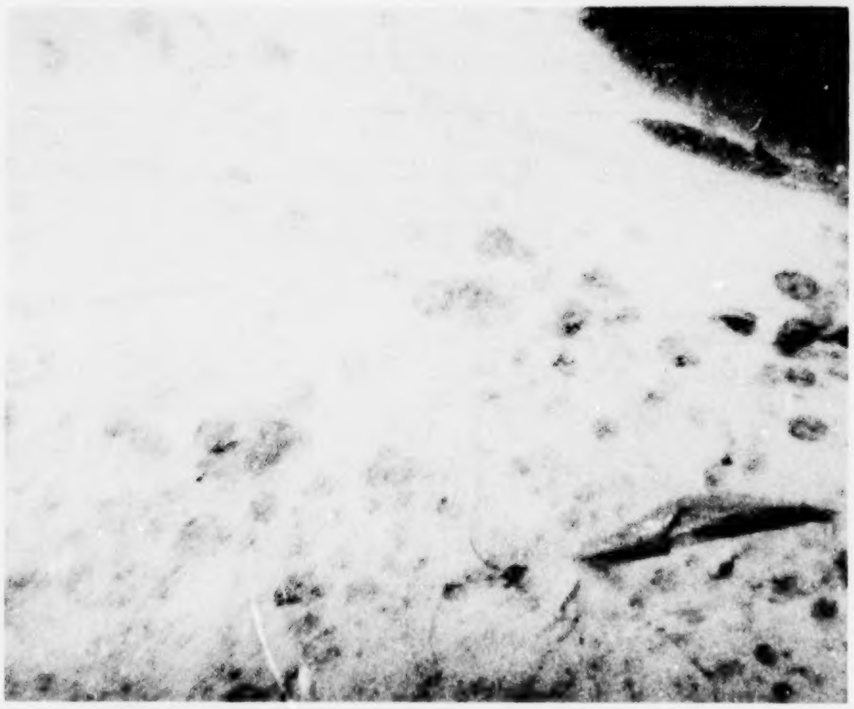


Figure 2. Subcutaneous Hemorrhages.

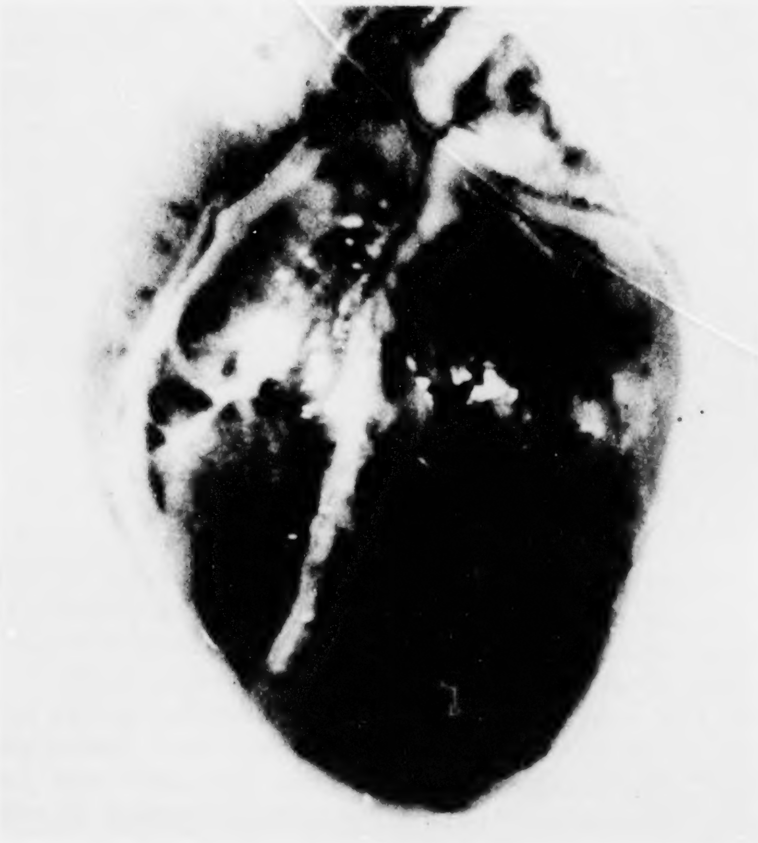


Figure 3. Heart Showing Ecchymotic Hemorrhages.

Figure 4. Extensive Petechial Hemorrhages. Serosa of Small Intestine.

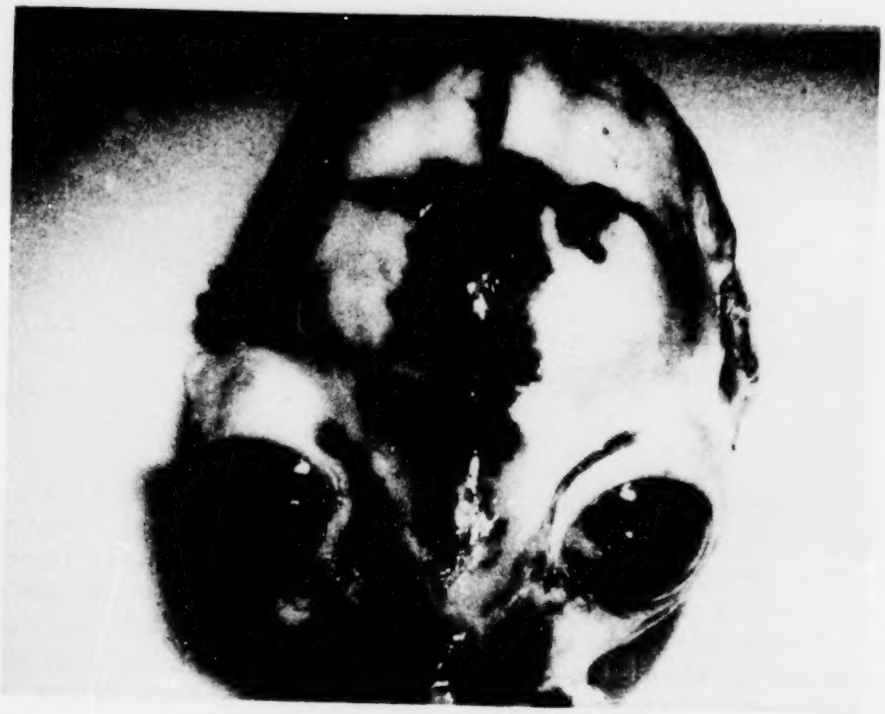


Figure 5. Skull Showing Hemorrhages along Cranial Suture Lines.

Bone marrow in most cases was bright red and semisolid in consistency; in some cases actual hemorrhage was evident in the marrow cavity.

Thymic atrophy was apparent in most cases and often ecchymotic hemorrhages were present in the gland and adjacent loose connective tissues.

Microscopic examination of tissue from these cases confirmed the gross findings of a generalized hemorrhagic condition. In addition to the areas of obvious gross involvement, hemorrhages were seen microscopically in such diverse sites as the lung, kidney, adrenal, caecum, and mesentery. Destruction of lymphoid tissue was not evident except as reflected by the varying amounts of thymic atrophy observed.

Bone Marrow

Bone marrow biopsies were obtained on all animals prior to death and impression smears made directly from fresh marrow at necropsy. Samples of bone marrow for routine hematoxylin and eosin and special stains were taken in both Zenker's fixative and formalin.

Results of the myeloid:erythroid (M:E) ratios available at this time are given in Table 3.

TABLE 3. M:E RATIOS

Age Group	Dose Level, μCi/day			
	100	50	25	0
Weanling	0.34:1	0.4:1	0.2:1	1.5:1
Adolescent	0.65:1			
Adolescent	0.08:1			

In all cases a pronounced depression of granulocytic cells was obtained which was reflected in the peripheral blood values. Impression smears revealed that the granulocytic cells which were present occurred in isolated islands where little maturation was evident beyond the metamyelocyte stage. Erythropoiesis was prominent in marrows of the low-dose groups; rare abnormal erythrocyte precursors were present in 2 cases (Figure 6). Also plasmacytopoiesis was common, and various forms of plasma cells were seen (Figure 7). Megakaryocytic forms were very rare or absent in most marrow smears.

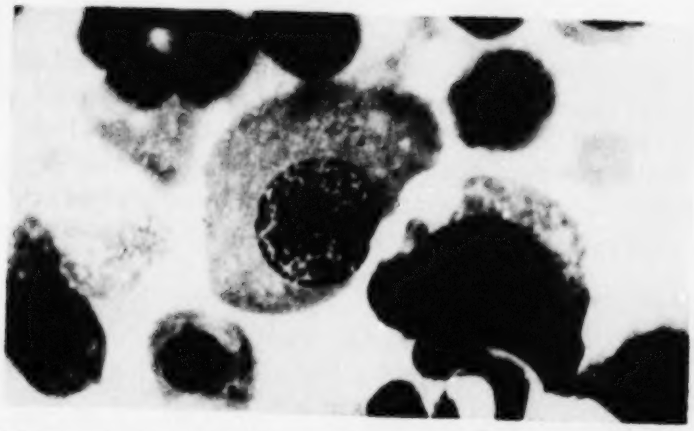


Figure 6. Giant Polychromatophylic Rubricyte in Bone Marrow.

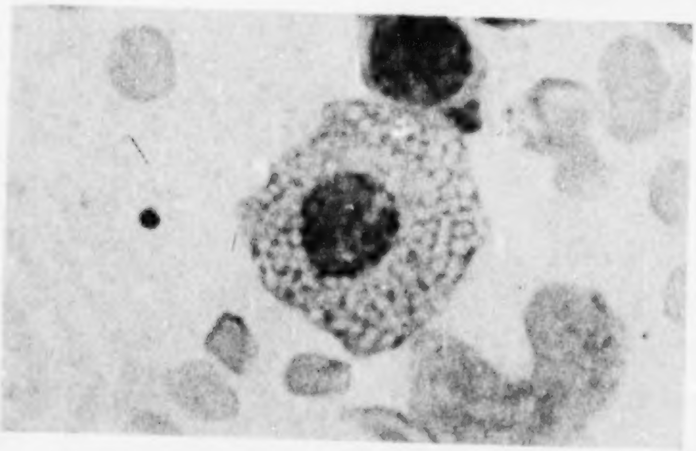


Figure 7. Plasma Cell with Russell Bodies in Bone Marrow.

Histopathological examination of Zenker's-fixed bone marrow revealed a gradation of marrow destruction related to the exposure dose level. Weanling cats on the 100 μCi level had aplastic, fatty marrows; weanling animals on the 50 μCi and 25 μCi levels and the adolescent animals on the 100 μCi level had moderately cellular to very cellular marrows (Figure 8).

Myelofibrosis (Figure 9) was seen in the bone marrows of three cats in one case as early as 12 days after cessation of isotope administration.

Ultrastructural studies of bone marrows of 4 cats from this pilot study are discussed under the Pathologic Studies Section of this report, p. 197.

Conclusions

Within age groups a marked dose response relationship was seen with regard to formed elements of the peripheral blood, bone marrow, and the radiation-induced hemorrhagic syndrome. However due to the rather marked differences in body burden accretion by various age groups, single uniform daily doses may be acutely toxic to some groups and produce little observable effects in others. The obvious conclusion from the pilot study, which is applicable to a basic experimental design, is that doses must be calculated not only on the basis of skeletal weight, but also on rates of body burden accretion because of the significant variation among age groups. Only in this manner can relatively similar skeletal burdens be produced in animals at varying stages of bone growth.

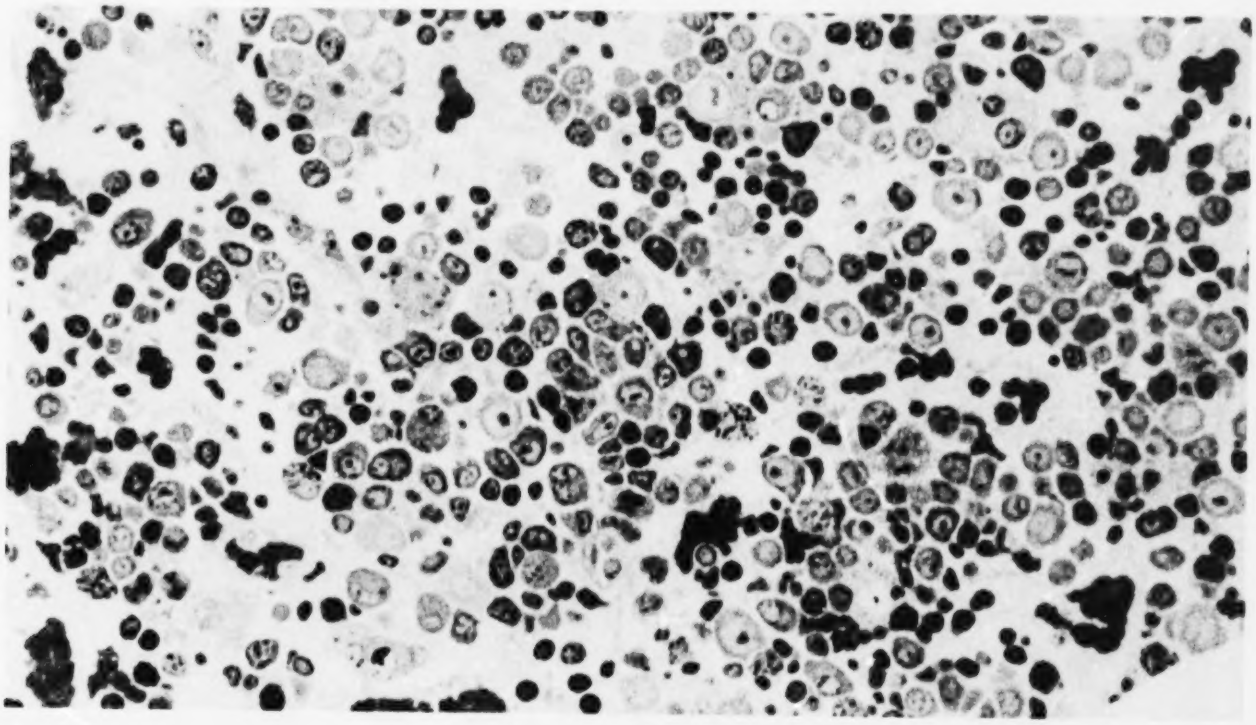


Figure 8. Bone Marrow with Pronounced Erythropoiesis and Plasmacytopoiesis. Epon-Embedded, Thick Section, Toluidine Blue Stain.

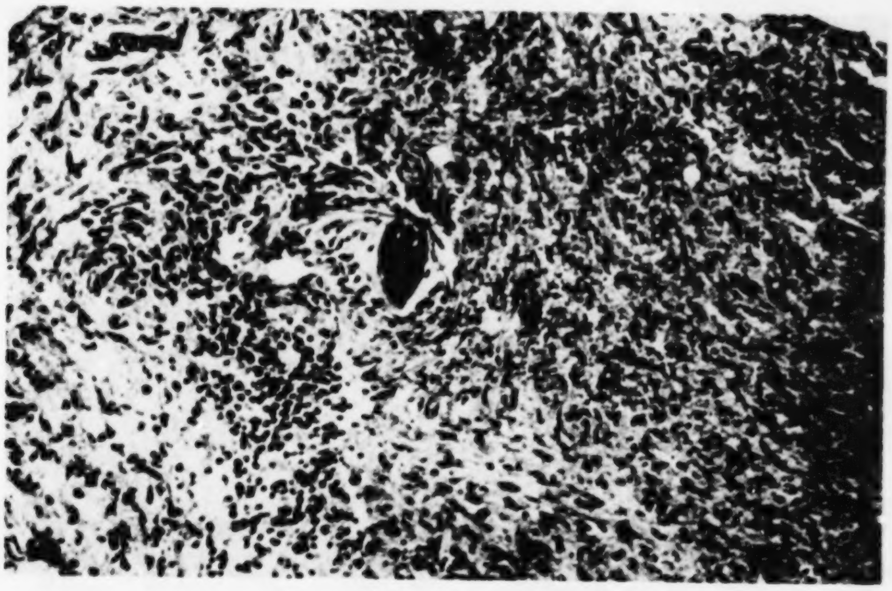


Figure 9. Bone Marrow Showing Myelofibrosis.

B. NEW FACILITIES AND TECHNIQUES

Safety Addition

C. G. Liddle

A safety addition to the colony maintenance building has recently been completed (Figure 1). The addition is designed to provide total support for the radioisotope exposure run so exposed animals can be maintained separately from the rest of the colony, and all procedures involving the use of radioisotopes in the animals can be carried out in one area. The only entrance to the exposure area will be through the new addition. Individuals will enter through the change and shower rooms where they will change into special clothing. The change rooms open into a hallway which will contain a clothes washer and dryer for laundering the special clothing.

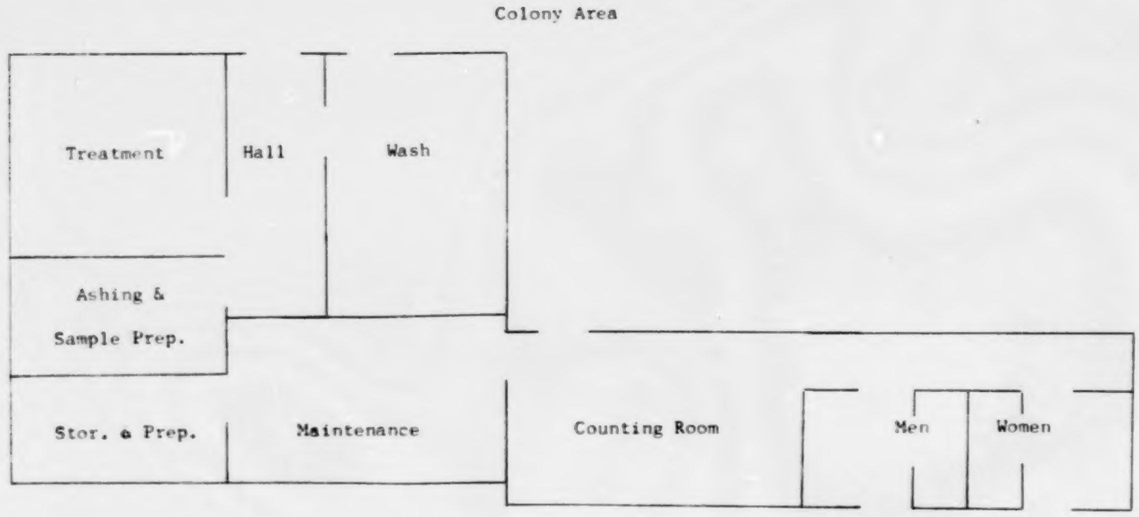


Figure 1. Colony Maintenance Building.

The hallway opens into the counting room which contains the cat whole-body counter. Next to the counting room is the maintenance room where exposed animals are examined and treated, radioisotopes are administered and other housekeeping chores are carried out for the exposure run. At the far end of the building next to the maintenance room is the radioisotope preparation and storage room. This room is equipped with a hood and a lead storage container for storing radioisotopes.

The preparation and storage room and maintenance room are part of the original maintenance building but have been divided from the rest of the building and made a part of the new addition for radioisotope control purposes.

The entire safety addition is temperature and humidity controlled. The floors are coated with an impervious material for ease of decontamination, and all floor-to-wall and floor-to-furnishing areas are calked for control of contamination. The floor drain and sinks empty into one of the radioactive waste water holding tanks.

Feline Whole-Body Counter

C. G. Liddle and K. E. Hawkins

The new feline whole-body counter consists of two opposing 5- by 2-inch crystals in 3-inch-thick cylinders of lead shielding. The crystals and shielding are mounted on steel plates and ride on roller bearings on steel rails allowing movement of the assemblies from a few centimeters to more than 2 meters from the center (Figure 2).

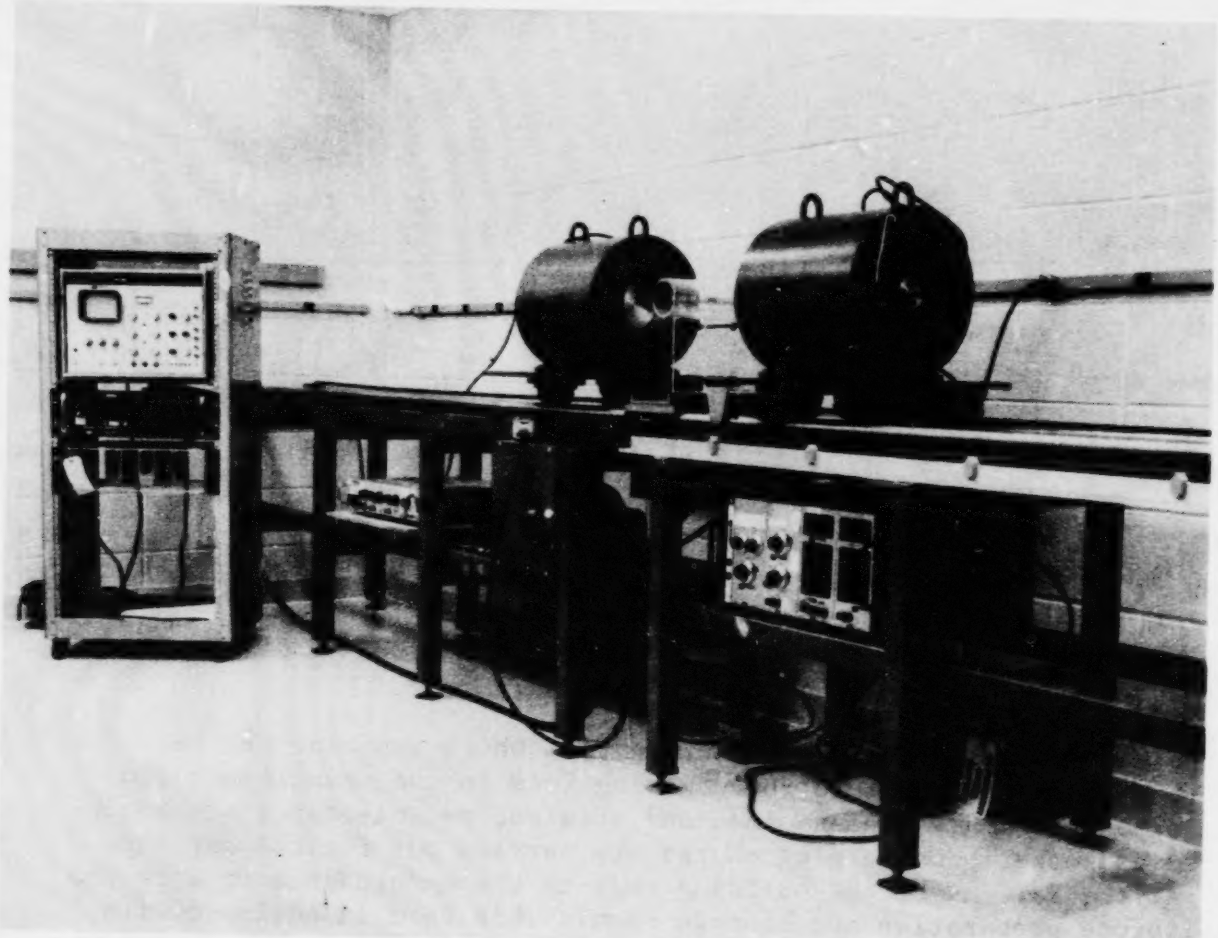


Figure 2. Whole-Body Counter. The feline is held in the lucite restrainer. Opposing crystals detect body burden which is recorded by electronic equipment for future analysis.

The detector assemblies are driven by an electric motor and a chain drive which operate the detectors at a speed of about 28 inches per minute. The motor is equipped with an electromagnetic brake allowing the assemblies to be stopped virtually instantaneously when the stop relay is activated with the control switch. Microswitches are located at each end of the rail to provide an automatic stop at the end of the travel in both directions. Also, a microswitch is mounted on one detector, and moveable stops to activate the microswitch are located on a bar alongside one rail. This allows automatic repositioning of the detectors at preset distances and provides a more reliable means of reproducing different detector distances than by stopping the assembly with the manual control.

The counter was delivered in November and is now being tested and calibrated. It will be used for counting gamma rays from cats receiving strontium-85 and bremsstrahlung x rays from strontium 89. From preliminary data it appears that counting efficiencies will be on the order of three times the efficiency of the single crystal now being used for whole-body counting of the cats.

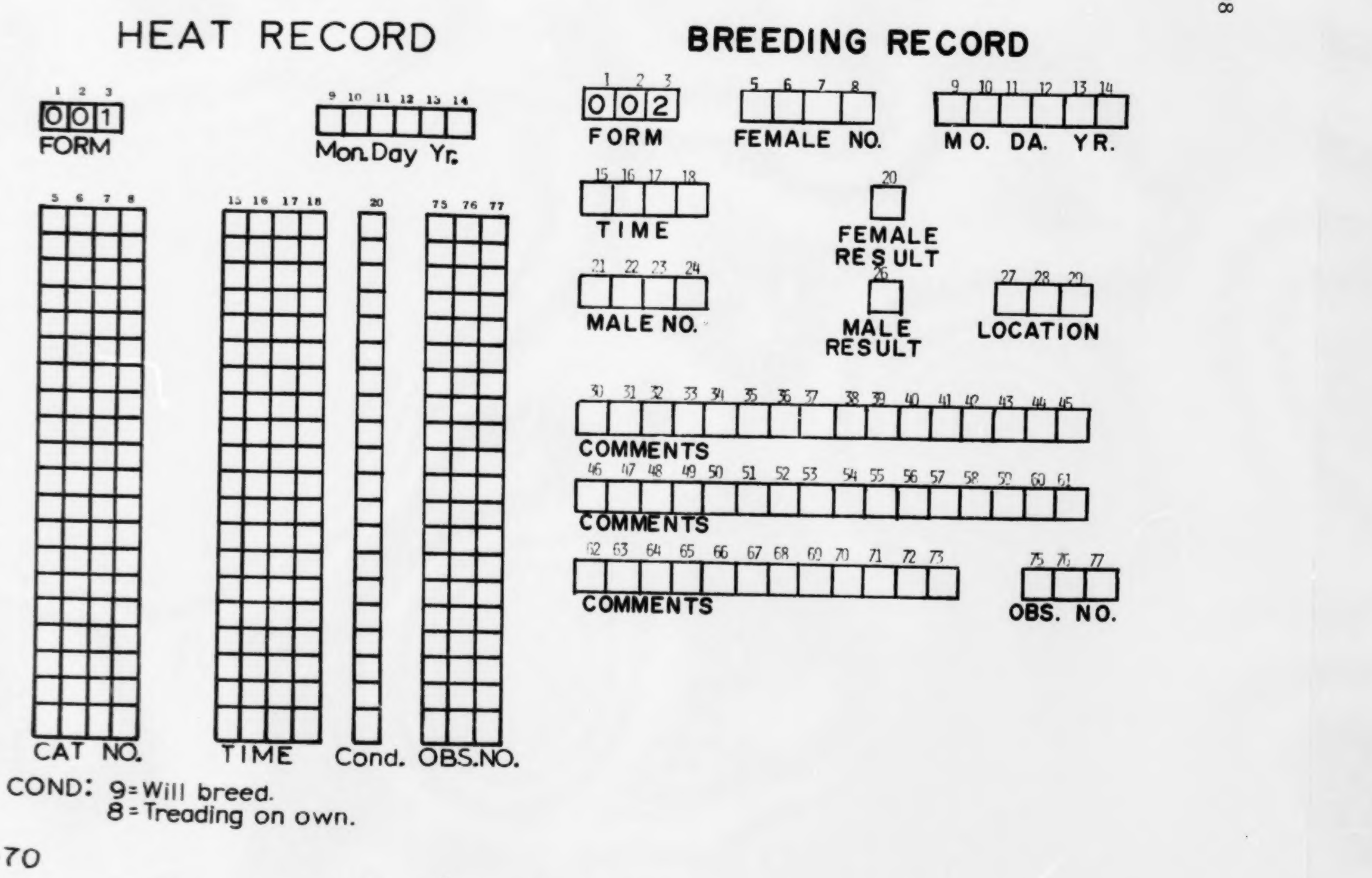
Data Handling System

L. Rosenstein and C. G. Liddle

In a long-term study involving a relatively large number of animals, one can expect to acquire a vast amount of experimental information over the lifetime of the experiment. In general, the mass of data will become so voluminous and unwieldy that without some sort of computer support, proper data handling will not be possible. Being aware of this situation and based on the experience of other laboratories involved in long-term studies, a teleprocessing link to facilitate data acquisition and retrieval has been established between the laboratory site and the central processing facility.

At the present time, all data are collected on acquisition forms designed by the individual investigators. Examples of the types of form designed and utilized by the Toxicologic Studies Section are shown in Figure 3. Once data have been entered on the forms, they are scanned for overt errors, corrected if necessary, and then submitted for entry into the appropriate computer file via the teleprocessor.

The teleprocessing terminal (Figure 4) consists of an IBM 1052 Printer-Keyboard and a 1051 Control Unit. This system is currently linked to an IBM 1058 Printing Card Punch. Thus, data entered at the laboratory appear as punched cards at the central processing facility. These cards are used to update the computer files now maintained on magnetic tape.



HEAT RECORD

1 2 3 FORM 0 0 1

9 10 11 12 13 14 Mon. Day Yr.

5 6 7 8 CAT NO.

15 16 17 18 TIME

20 Cond.

75 76 77 OBS. NO.

COND: 9 = Will breed.
8 = Treading on own.

7-70

BREEDING RECORD

1 2 3 FORM 0 0 2

5 6 7 8 FEMALE NO.

9 10 11 12 13 14 MO. DA. YR.

15 16 17 18 TIME

20 FEMALE RESULT

21 22 23 24 MALE NO.

26 MALE RESULT

27 28 29 LOCATION

30–45 COMMENTS

46–61 COMMENTS

62–73 COMMENTS

75 76 77 OBS. NO.

Figure 3. Examples of Data Acquisition Forms Designed and Used by the Toxicologic Studies Section.

All data entered via the 1051/1058 link undergo edit checks before they are added to the proper computer file. Those data found to contain overt keypunch errors are repunched. Data records that survive the edit checks are filed and their authenticity is checked the next time the particular file is dumped for examination. Once the pertinent portion of the file has been verified, the original source documents are microfilmed and then destroyed.

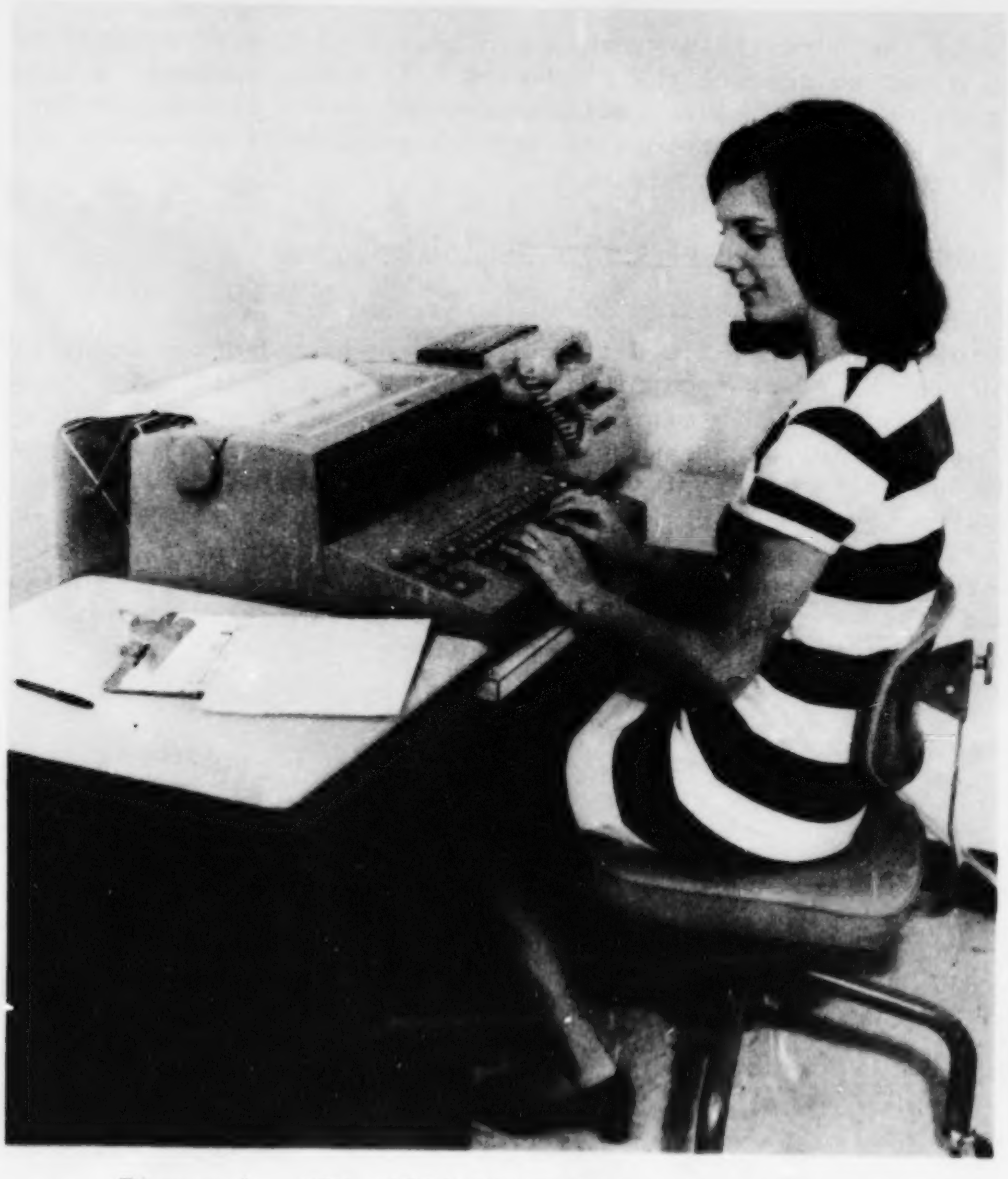


Figure 4. IBM 1051/1052 Teleprocessing Unit.

Epoxy Resins for Embedding Undecalcified Bone

C. Miller

This laboratory is seeking a suitable medium, other than methacrylates, for embedding and sectioning (10 microns) undecalcified bone. Current experiments are with Durcupan ACM (FLUKA AG, Buchs SG, Switzerland), an Araldite (CIBA, Basel, Switzerland) base embedding agent. The procedure requires dehydrating bone sections (marrow removed) through alcohols (including defatting with alcohol-ether) and infiltration with modified solutions of Durcupan ACM (epoxy resin-hardener-dibutyl phthalate).

The formula for Durcupan mixture 2 provides a choice of amounts of accelerator to use to control the hardening. To date, however, a block of satisfactory hardness has not been produced. Tests are continuing to determine the exact proportions of various components necessary and to provide means for thorough mixing.

Histopathological Evaluation of Epon-embedded Sections

J. M. Ward

Bone marrow and kidney, fixed in glutaraldehyde and osmic acid, are being embedded in Epon, an epoxy resin usually used for ultrastructural studies. Sections, 0.5 to 1 micron thick, are cut with a glass knife and stained with a solution of steaming toluidine blue. Resolution in the light microscope is considerably better than that obtainable with paraffin-embedded tissues (Figure 8, p. 94). The 0.5 to 1 micron thick sections will be studied and compared with sections prepared from paraffin-embedded tissues, tissue smears, and imprints and electron micrographs. The same Epon-embedded tissues are also sectioned at 50 millimicrons with a diamond knife for examination in the electron microscope.

C. STRONTIUM-85 STUDIES

Strontium-85 Uptake and Distribution Study

Neal S. Nelson

Thirty felines of various specific ages have been orally dosed with 0.5 μCi ^{85}Sr/day for a 65-day period. Whole-body radioassay counts were made during the 65 days to define the uptake curves. Eighteen of these animals have been sacrificed, each dissected into 78 component organs and bones; the components have been weighed, assayed for ^{85}Sr content, and ashed. The data from the study have not yet been analyzed.

Embryonic Uptake of Strontium 85

Neal S. Nelson and Joan Cooper

The purpose of this study is to obtain strontium 85 uptake curves for embryonic felines.

Litters are obtained from dams that were continuously dosed with 0.5 μCi ^{85}Sr/day. The queens are not bred until they are on a plateau with a constant body burden for at least 3 months. Embryos, placentae, and fluids are obtained by embryotomy, weighed, and assayed for strontium-85 content.

Curves for total strontium-85 content and the activity per gram of tissue are shown for both embryos and placentae (Figure 1). The curves for embryo total activity and activity per gram have two periods of extremely heavy deposition of strontium 85. The first of these periods is during the 4th week of gestation. Rapid deposition of bone-seeking isotopes would be expected at this time, since as shown by stable chemistry analyses and alazarin red staining, bone mineral deposition starts between the 4th and 5th week of gestation. A second period of bone mineralization evidently occurs during the last few days of the gestation, with about 50 percent of the neonatal body burden being deposited in the last two days of gestation.

Total activity in the placenta suggests that there is very rapid growth of the placenta from nidation at 10-14 days until the 5th week of gestation, then the placental mass is constant. The rather erratic but constant activity per gram further suggests that the change in total placental activity is due to a change in mass rather than function. A weight curve shown elsewhere tells the same story of nearly constant mass after the 5th week of gestation.

Twenty-two embryos of 7, 8, or 9 weeks gestation have each been dissected into 72 individual organs or bones to determine the organ weight and strontium-85 distribution. Dissections of embryos will be extended to as early a gestational period as is practical. Analysis of these data requires a computer program which is not fully operational, so no report can be made on distribution at this time.

Additional animals have been scheduled into the study so that each point will represent a minimum of three complete litters. The only problems anticipated are at 2 weeks of gestation or earlier since this is near or before nidation and the ova will be difficult to locate.

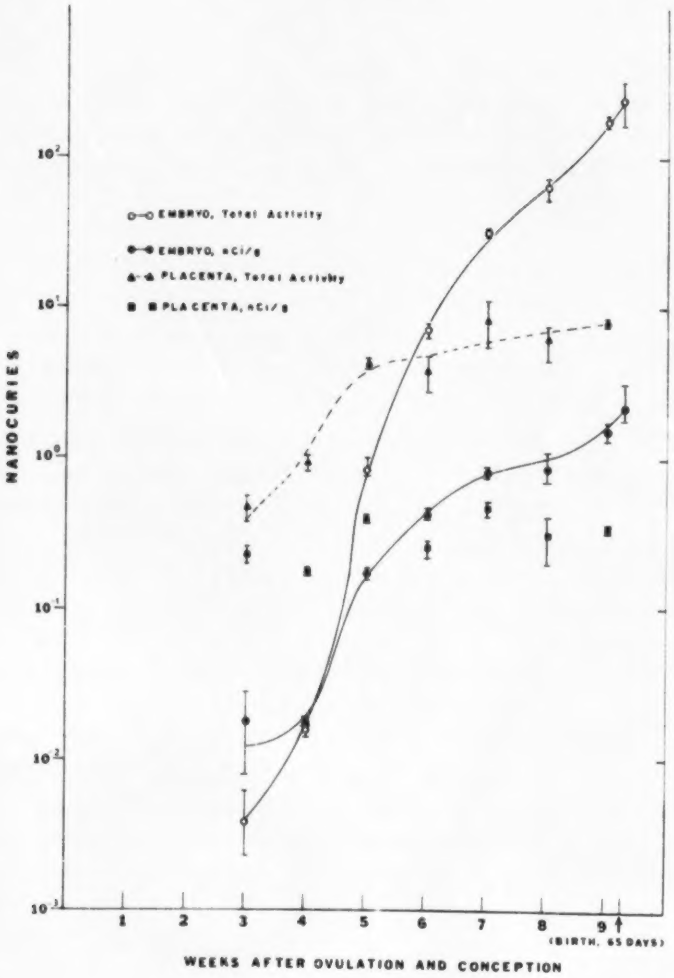


Figure 1. Strontium-85 Activity in the Embryo and Placenta.

D. A PRELIMINARY REPORT ON IRON-59 DISTRIBUTION IN THE FELINE

N.S. Nelson, J. Parrish and H. Barkau

Radioiron deposited in the skeletal system is considered in this study to be deposited in areas of active bone marrow. Strontium 85 was used in conjunction with iron 59 in order to investigate the proportion of deposited radiostrontium in close proximity to active bone marrow sites. Additional data of this nature should help to extend and clarify determinations of deposited radiostrontium distribution in felines.

A 9-month-old male cat was injected with 8 μCi of iron 59 and 6 μCi of strontium 85. Whole-body counts and blood samples were obtained at 0, 2, 4, 6, and 24 hours postinjection. After the 24-hour samples were taken, the animal was sacrificed by exsanguination (under anesthesia) and dissected. All tissues obtained were assayed for both iron-59 and strontium-85 content.

Results and Discussion

As shown in Table 1 and Table 2, more than half of the iron 59 (63.5 percent) is retained in the skeleton of the cat 24 hours after dosing. About 40 percent of this iron is in the vertebrae and 36 percent in the long bones of the appendages with the remaining 24 percent distributed primarily in the skull, scapulae, pelvis, and ribs.

Strontium 85 is deposited primarily in the skeleton (82.5 percent) with the scapula, radius, and fibula having the highest rate of uptake for the 24-hour period.

TABLE 1. ^{59}FE and ^{85}SR DISTRIBUTION IN THE ORGAN SYSTEMS OF AN ADULT CAT 24 HOURS AFTER INTRAVENOUS DOSE

System	% of Body Weight	% of Sacrifice Body Burden	
		^{85}Sr	^{59}Fe
Integumentary	16.404	2.3428	2.8011
Respiratory	0.638	0.1578	0.8074
Gastrointestinal	5.364	7.2841	6.2650
Urogenital	0.730	0.0781	0.0545
Central Nervous	1.297	0.1769	0.0975
Skeletal	9.259	82.4550	63.4382
Internal Organ	3.953	0.9596	10.9675
Muscle and Connective Tissue	55.306	5.7719	9.9653
Mesentery and Omentum	2.592	0.0681	0.2852
Blood	4.456	0.7054	4.4831

TABLE 2. ^{59}Fe AND ^{85}Sr DISTRIBUTION IN THE TISSUES OF AN ADULT CAT 24 HOURS AFTER INTRAVENOUS INJECTION

SYSTEM	% OF BODY WEIGHT	% OF SACRIFICE BODY BURDEN		% OF SACRIFICE BODY BURDEN PER GRAM	
		^{85}Sr	^{59}Fe	^{85}Sr	^{59}Fe
CENTRAL NERVOUS SYSTEM					
Eyes	0.276	0.0615	0.0081	0.00608	0.00080
Spinal Cord	0.251	0.0295	0.0228	0.00320	0.00246
Brain	0.770	0.0859	0.0665	0.00304	0.00234
INTEGUMENT					
Skin, Hair	16.366	2.2726	2.7975	0.00378	0.00464
Nails	0.038	0.0718	0.0036	0.05135	0.00256
RESPIRATORY					
Lungs	0.483	0.0623	0.7444	0.00351	0.04180
Trachea	0.155	0.0955	0.0630	0.01682	0.01106
GASTROINTESTINAL					
Tongue	0.259	0.0328	0.8060	0.00344	0.00908
Esophagus	0.134	0.0161	0.0330	0.00338	0.00670
Stomach (With Contents)	0.615	0.0802	0.3614	0.00355	0.01594
Duodenum (With Contents)	0.463	0.0337	0.5331	0.00198	0.03125
Jejunum (With Contents)	0.704	0.0585	1.0650	0.00226	0.04102
Ileum (With Contents)	1.277	0.1626	2.1972	0.00347	0.04669
Colon (With Contents)	1.507	6.6407	1.8970	0.12000	0.03415
Rectum (With Contents)	0.405	0.2589	0.0916	0.03698	0.00613
UROGENITAL					
Kidneys	0.545	0.0666	0.8212	0.00333	0.04086
Bladder	0.071	0.0110	0.0119	0.00453	0.00453
Testes	0.081	- ----	0.0497	- -----	0.01664
Penis	0.033	0.0781	0.0060	0.00291	0.00499

ORGANS					
Heart	0.316	0.5464	0.3986	0.04705	0.03419
Liver	2.786	0.3131	8.7166	0.00306	0.08488
Spleen	0.146	0.0102	1.1598	0.00190	0.21550
Thyroid	0.011	- ----	0.0047	- -----	0.01194
Adrenals	0.009	0.0005	0.0052	0.00150	0.01638
Pancreas	0.221	0.0246	0.4705	0.00304	0.05777
Parotid	0.114	0.0180	0.0038	0.00430	0.00092
Mandibular	0.072	0.0217	0.0043	0.00824	0.00161
Zygomatic	0.014	0.0098	0.0051	0.01842	0.00955
Lymph Nodes (All)	0.082	0.0070	0.0466	0.00233	0.01540
Thymus	0.184	0.0083	0.0792	0.00123	0.01172
Omentum & Mesentery	2.592	0.0681	0.2852	0.00072	0.00298
BLOOD					
RBC, Plasma	4.456	0.7054	4.4831	0.00429	0.02729
MUSCLE	55.306	5.7719	9.9653	0.00284	0.00489
SKELETAL					
Skull	1.255	11.595	2.0651	0.25171	0.04466
Mandible	0.255	2.410	0.1453	0.25733	0.01545
Vertebrae, Cervical	0.385	3.148	5.8831	0.22285	0.41489
Thorasic	0.599	5.264	9.3650	0.23920	0.42395
Lumbar	0.695	6.692	10.4576	0.26232	0.40834
Sacral	0.107	0.986	1.0914	0.25087	0.27656
Coccygeal	0.281	2.113	0.3566	0.20437	0.03438
Scapula	0.290	3.673	1.7743	0.34463	0.16585
Humerus	0.722	6.759	7.6120	0.25493	0.28601
Radius	0.233	2.997	0.8114	0.34978	0.09435
Ulna	0.287	2.601	1.0662	0.24658	0.10069
Carpals; Metacarpals, (F) Phalanges	0.546	2.612	0.0739	0.13029	0.00367
Pelvis	0.462	4.772	5.1339	0.28113	0.30132
Femur	0.824	7.781	8.4525	0.25720	0.27832
Tibia	0.670	7.011	4.2702	0.28490	0.17290
Fibula	0.085	1.092	0.1571	0.34959	0.05012
Tarsals, Metatarsals, (R) Phalanges	0.945	6.606	0.1213	0.19030	0.00348
Ribs	0.540	3.793	3.6139	0.19150	0.18174
Sternum	0.077	0.546	0.9869	0.19206	0.34589

Radioisotope distributions within the five major vertebral divisions, within the long bones, and within the ribs, scapulae, and pelvis are presented in Figures 1, 2, and 3.

Of particular interest are iron-59 and strontium-85 distribution patterns in the vertebrae and long bones in that these bones represent a major proportion of deposited skeletal radioisotopes.

More iron 59 (Figure 1a) is concentrated in the axis than in the remaining cervical vertebrae, although strontium 85 is distributed relatively uniformly within this group. In comparison, iron-59 distribution shows a tendency to fluctuate within the thoracic (Figure 1b), and lumbar (Figure 1c) vertebrae; additional investigation with respect to the significance of these variations may further clarify the iron-59 vertebral distribution pattern.

It may be noted in Figure 1e that iron-59 concentration becomes negligible in regions caudal to the first three coccygeal vertebrae, while the strontium-85 distribution in these regions appears to follow a reverse trend.

Deposited iron 59 in the humerus (Figure 2a) appears to concentrate primarily in the proximal and distal regions, with strontium-85 distribution following a similar trend. The radioiron concentration in the humerus is maximum in the area immediately distal to the tubercular region. Iron 59 in the radius and ulna (Figures 2b, 2c) appears to be proportionately higher in the most proximal regions, while a comparatively greater strontium-85 concentration is observed in the most distal regions of these bones.

Radioisotope distribution patterns in the femur (Figure 2d) parallel those observed in the humerus. Although iron-59 distribution in the fibula and tibia (Figures 2e, 2f) appears to be somewhat similar to that in the radius and ulna, there is a relatively greater concentration of strontium 85 in the proximal regions of the fibula and tibia. The strontium-85 distribution trend in these bones appears to be quite similar to that observed in the femur.

Of the remaining tissues, the spleen with 1.2 percent, the skin with 2.3 percent, the blood with 4.5 percent, the liver with 8.7 percent, and the muscle with 10 percent had the highest proportions of iron 59. The proportion (~10 percent) of the injected radioiron found in the liver and spleen is less than the 50 percent concentration reported for beagles (1) and closer to the 20 percent concentration reported in humans (2); however, more animals must be assayed to be sure of the values. If all of the iron 59 injected in the cat is considered to be in sites of active bone marrow, then the distribution of marrow in the cat can be compared with the rat and with man (Table 3). The distribution of marrow in the cat is

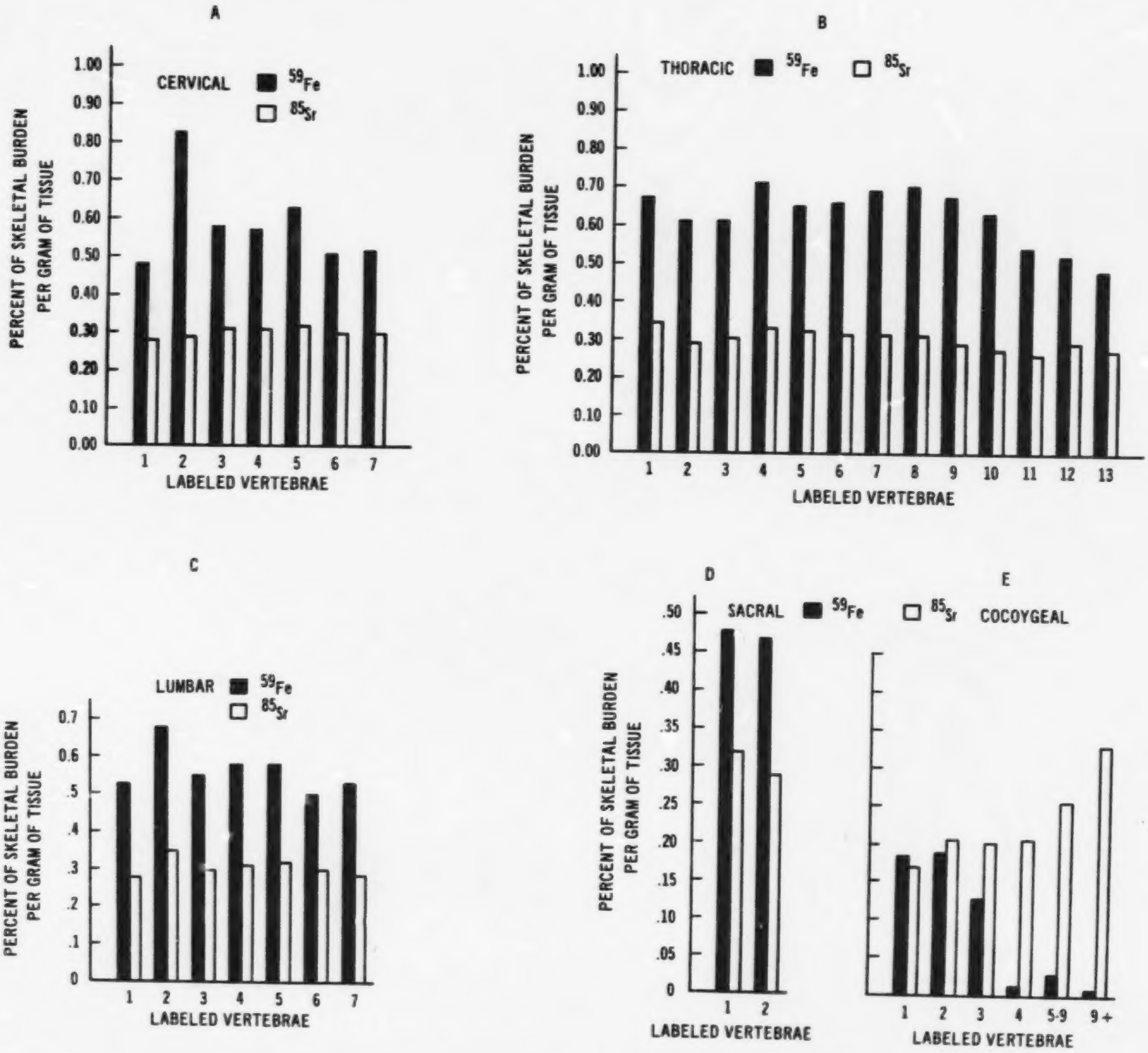


Figure 1. Iron-59 and Strontium-85 Distribution in Various Bone Groups.

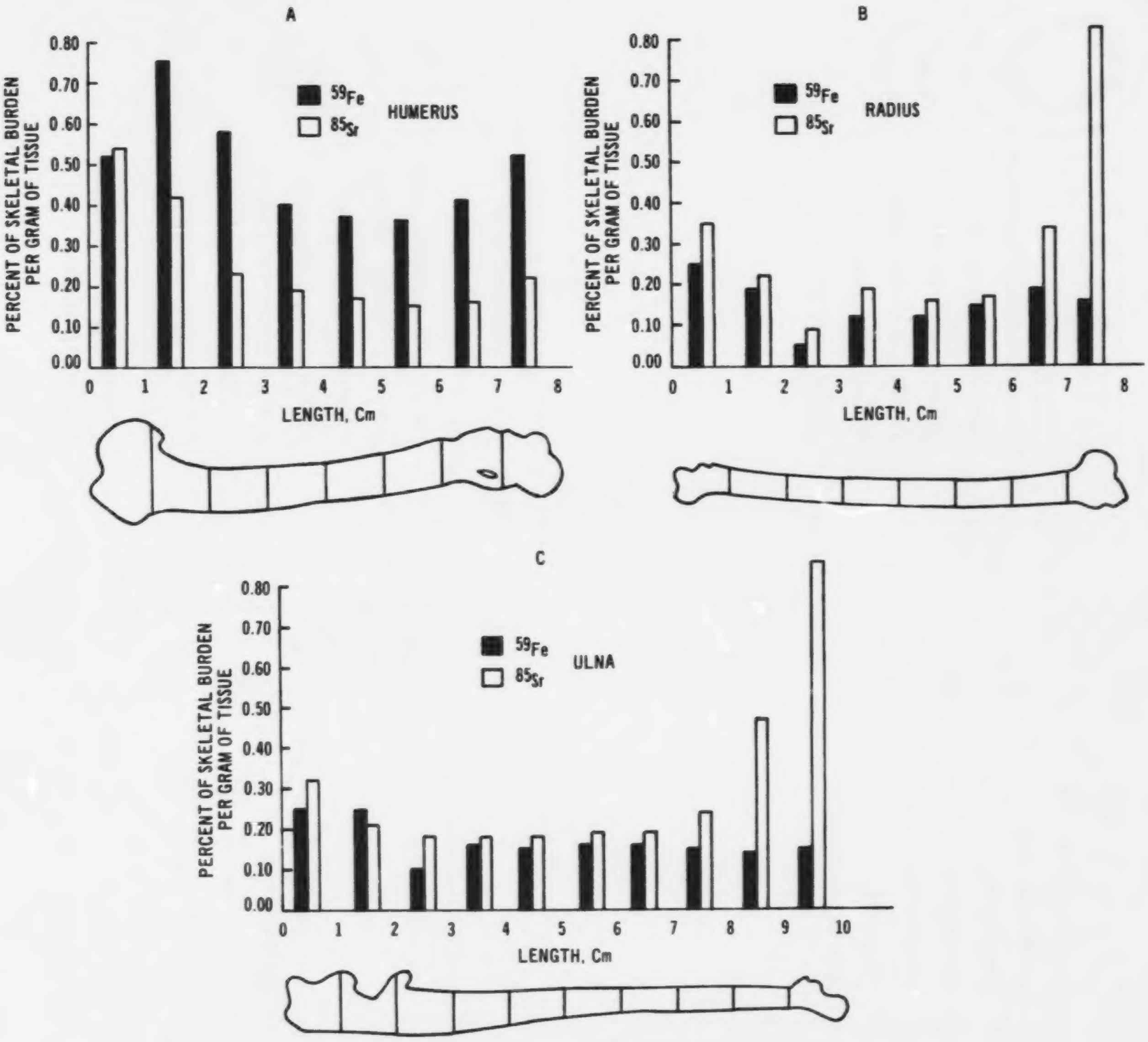


Figure 2. Iron-59 and Strontium-85 Distribution in Long Bones.

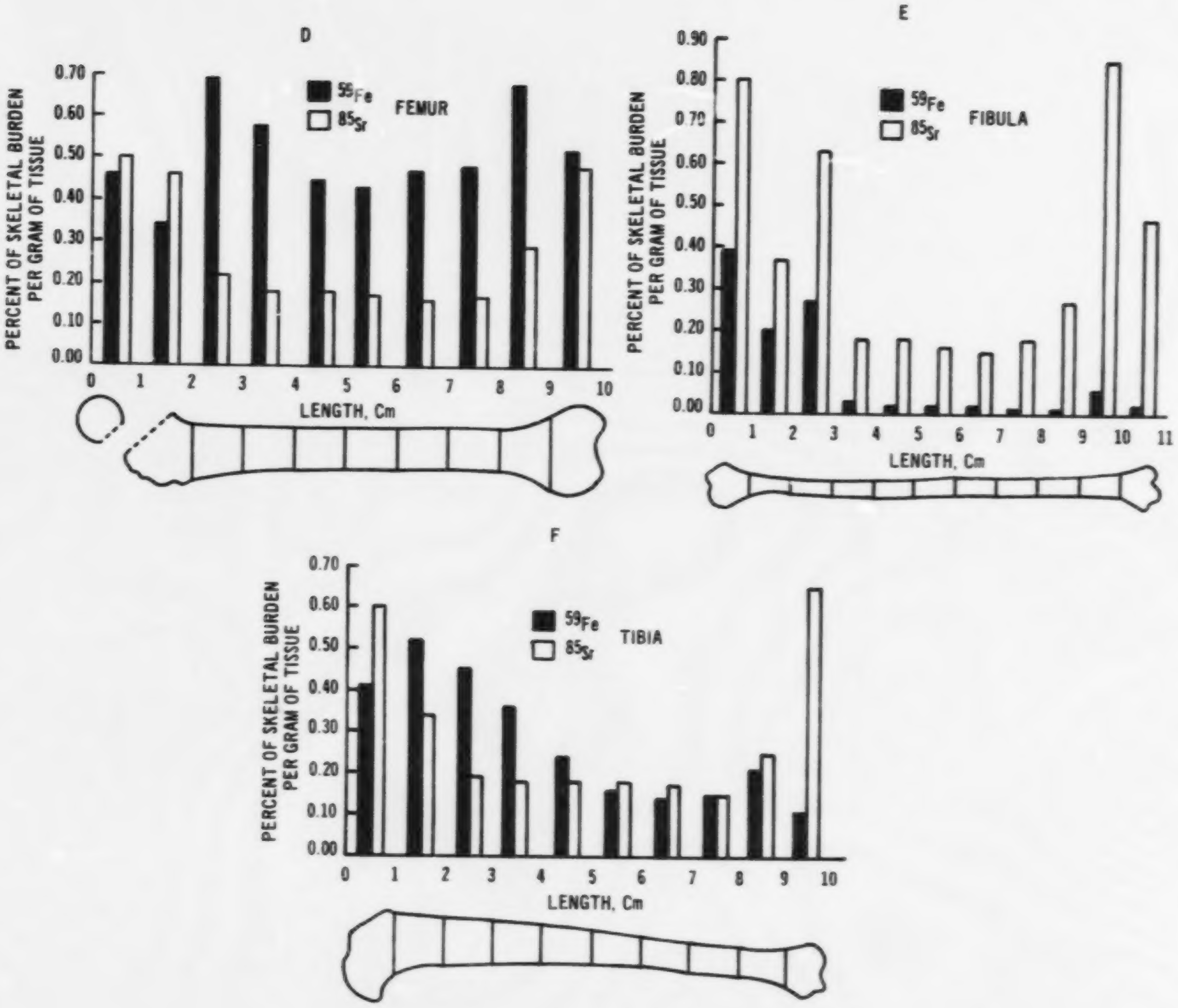


Figure 2. Continued.

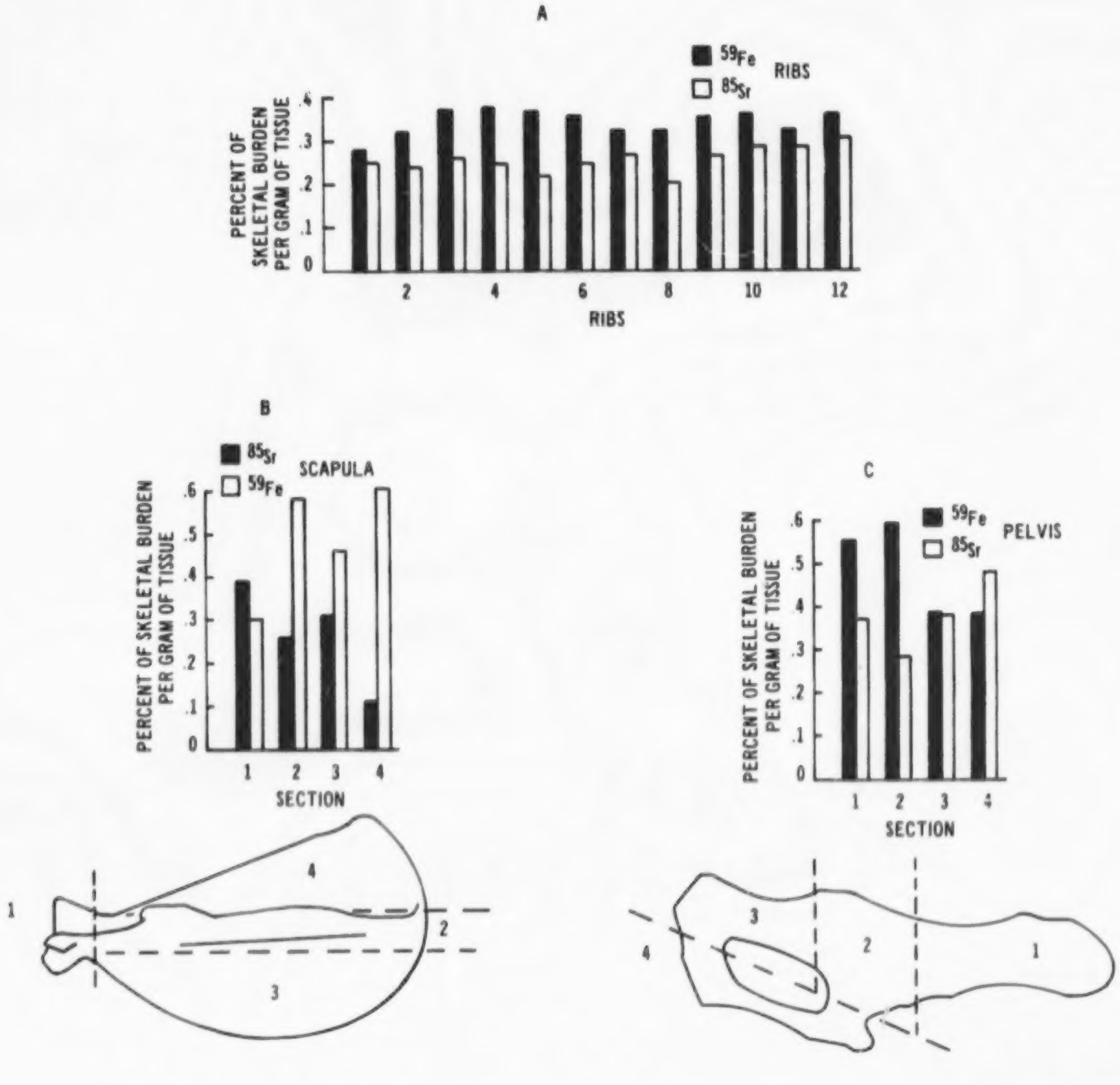


Figure 3. Iron-59 and Strontium-85 Distribution in Flat Bones.

similar in some respects to both man and rat but there are significant differences. The cervical vertebrae in the cat appear to concentrate appreciably more and the caudal vertebrae less radioiron than either of the other species. Further, the sacrum has a much lower iron-59 level than would be expected from the figures reported for either rat or man. These differences in distribution of marrow activity are important to the calculation of rad dose to active marrow and will be investigated in greater depth.

TABLE 3. PERCENT DISTRIBUTION OF
BONE MARROW IN THE CAT, RAT, AND MAN

Bone		Cat[a]			Rat[b,c]		Man			
							Marrow Spaces[d]		Red Marrow[e]	
Skull	(entire)	3.26	3.49		4.07	6.65		6.33		13.1
Mandible	(entire)	0.23			2.58			0.60		
Vertebrae	(entire)	42.79			35.44					42.3
Cervical	(7)[f]	9.27		(7)	2.41		(7)	1.78	(7)	3.4
Thoracic	(13)	14.76		(13)	9.88		(12)	7.29	(12)	14.1
Lumbar	(7)	16.48		(4)	7.60		(5)	5.61	(5)	10.9
Sacral	(2)	1.72		(5)	9.80			-	(5)	13.9
Caudal	(21)	0.56		(30)	5.75			-		-
Scapula	(1)	1.40	7.40		0.77	4.25		1.19		4.2
Humerus	(1)	6.00			3.48			2.99		
Radius	(1)	0.64			0.20			0.69		-
Ulna	(1)	0.84			0.51			0.67		-
Front Foot	(1)	0.06			0.10			1.37		-
Pelvis	(1)	4.05	10.71		4.17	12.62		8.14		13.0
Femur	(1)	6.66			8.45			8.53		
Tibia	(1)	3.37			5.66			5.46		-
Fibula	(1)	0.12			0.10			0.77		-
Hind Foot	(1)	0.10			0.18			4.21		-
Ribs	(13)	5.70		(13)	6.22		(12)	7.34	(12)	7.9
Sternum	(entire)	1.56			4.12			1.38		2.3

a. Data obtained 24 hours after ^{59}Fe injection.

b. Data obtained 5 hours after ^{59}Fe injection.

c. Van Dyke, D., Anger, H., and Pollycove, M. 1964. The effect of erythropoietic stimulation on marrow distribution in man, rabbit and rat as shown by Fe^{59} and Fe^{52}. Blood 24:356-371.

d. Woodard, H.Q., and Holodny, E. 1960. A summary of the data of Mechanik on the distribution of human bone marrow. Phys. Biol. Med. 5:57-59.

e. Ellis, R.E. 1960. The distribution of active bone marrow in the adult. Phys. Biol. Med. 5:255-258.

f. Numbers in parentheses indicate numbers of bones assayed.

The 24-hour blood curves showing disappearance of iron 59 and strontium 85 from the blood are shown in Figure 4. The curve of strontium-85 disappearance from the blood is comparable to other curves of the same type. The iron-59 curve has a rather rapid initial disappearance rate which later reverses so that by 24 hours after dosing, the curve has returned to the 4-hour level. This return might represent the start of a "hump" in the curve as reported in the plasma curve in man (2) or it may represent the first iron-59 labelled red cells appearing in the circulation. Further investigation of iron 59 in the circulating blood will be conducted across longer time spans and will include separation of samples into plasma and cells for radioassay.

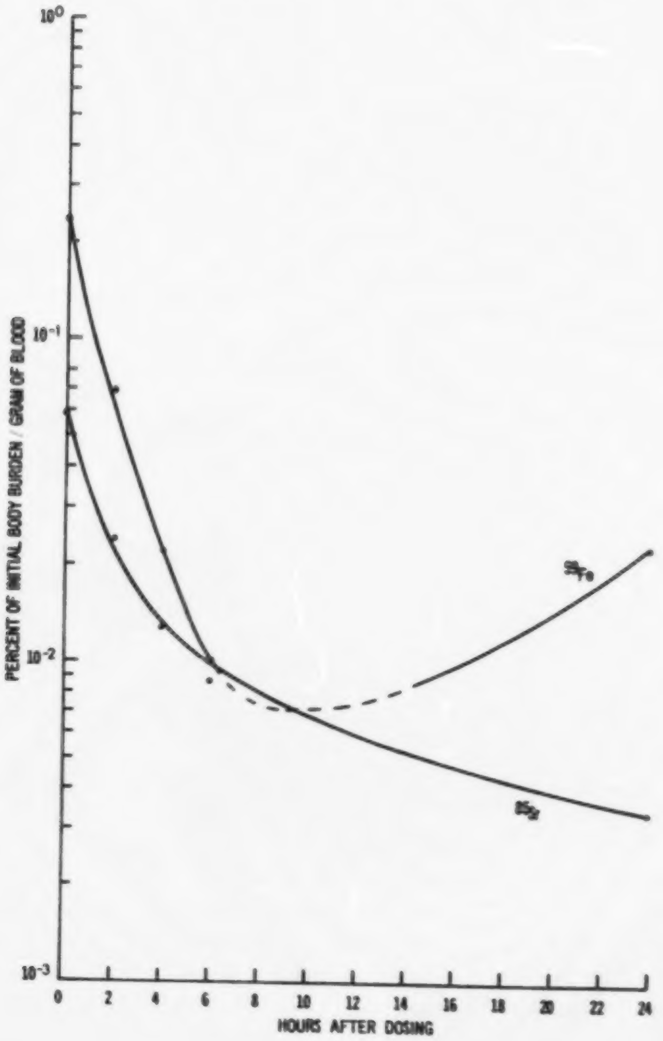


Figure 4. 24-Hour Disappearance Curves for ^{59}Fe and ^{85}Sr.

There is also suggestion from the data in Table 2 that endogenous excretion of iron 59 is less than 5 percent and this primarily from the lower small intestine. This also will be investigated further.

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1. University of California (Davis). 1969 Annual Report (UCD 472-116), p. 73.
2. Gevirtz, N.R., L.R. Wasserman, L. Sharney, and D. Tendler. 1965. Studies of plasma Fe^{59} disappearance - A manifestation of ineffective erythropoiesis and of hemolysis. Blood 25:976-989.

E. FELINE GROWTH AND DEVELOPMENT

Body Weight

L. Rosenstein and C. Altheide

In long-term toxicologic investigations, it is very often useful and necessary to define the normal growth characteristics of the experimental animals. The felines in the Toxicologic Studies colony are routinely weighed throughout most of their normal lifetime. The preliminary results of these serial weighings are shown in Figure 1. As more data become available, the trend of the curves will be re-evaluated, error terms added, and the curves themselves will be extended.

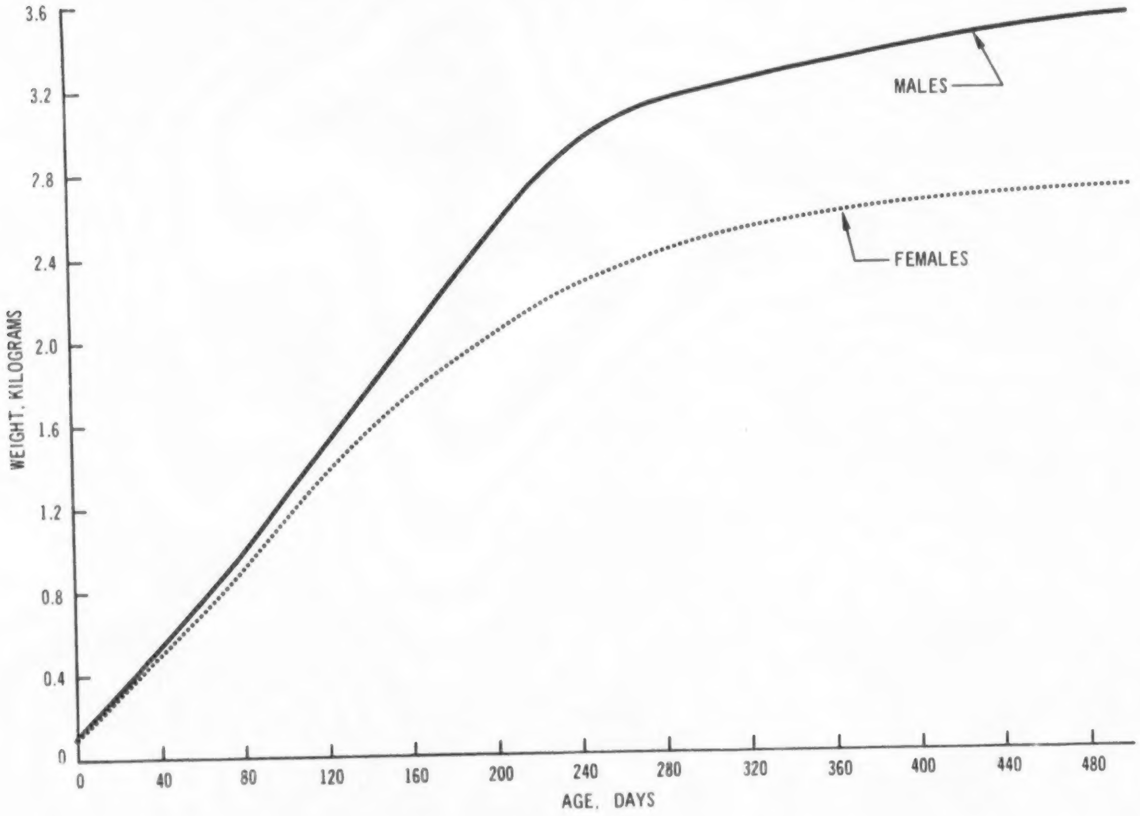

Figure 1. Average Weight Curves for Felines in the Toxicologic Studies Colony (Preliminary).

Microscopic Calcium Metabolism

L. Rosenstein, C. Miller

Calcium is added to bone by apposition (growth) and by augmentation (long-term exchange and secondary mineralization). In young animals, apposition is the predominant ion transfer process, whereas in older, more mature animals, augmentation tends to predominate. This shift in the predominant ion transfer process will influence the rate at which calcium is added to bone. Thus, it should be possible to define skeletal and hence chronological age on the basis of calcium addition rates. With this in mind, an experiment has been designed to measure the rate at which calcium is added to bone as a function of age.

Animals 1, 2, 4, 6, 8, 10, 12, and 18 months old will be needed for the study. Each animal will receive a marker dose of tetracycline on day 0. On day 6, calcium 45 will be administered intravenously. Commencing 1/2 hour postinjection, the animals will be serially bled to obtain a curve of serum specific activity. On day 10, another marker dose of tetracycline will be administered, and the animals will be sacrificed on day 12.

Before fully implementing the experimental design, a preliminary investigation was conducted to find and correct errors in the experimental technique and to obtain some idea of the magnitude of the calcium addition rates. Two animals were used in the study. Each received the multiple injections of tetracycline as well as the single injection of calcium 45 at the required time. Blood for serum preparation was collected 1/2, 1, 2, 3, 4, 6, 8 hours, and 1, 2, 4, 6, and 7 days post-calcium-45 administration. Serum samples then were radioassayed and prepared for stable calcium analysis. At sacrifice, appropriate bones were removed, embedded, and subsequently radioautographed.

Little difficulty was encountered in carrying out the experimental techniques. However, the quantitative results of the preliminary investigation have yet to be fully evaluated. Not all of the bone radioautograms have been photometrically analyzed and there still are a relatively large number of serum samples requiring stable calcium analysis.

Growth of the Feline Long Bones

E. Berman, B. Bolden, J. Parrish

In developing the experimental design for the strontium-89 core project, some method was needed to delineate the age groups to be studied and to distinguish between adolescence and maturity in felines. Skeletal maturation was selected as a partial criterion for such distinction, using appendicular radiographs of the feline long bones. Parameters under consideration include the rates of bone shaft growth and epiphyseal closure.

Samples of at least eight normal cats, four males and four females, are being obtained at each of several intervals during postconception (4-9 weeks); birth; suckling and weanling (2-15 weeks); young and maturing (4-18 months); and mature (21+ months). Serial sampling of several litters from birth and random sampling are being conducted.

Measurements of total bone length are being determined for the humeri, radii, ulnae, femura, tibiae, and os calces. The following bone lengths are being measured: Most distal styloid to most distal anconeal processes of the ulnae, and most distal surface to the lesser trochanter of the femura.

Epiphyseal closure is being observed in the distal humeri, distal radii, distal ulnae, distal femura, tibial tuberosities, and proximal os calces.

Bone length data obtained thus far are represented by the total bone length determinations of the femur and os calcis (Figure 2). The maximal increase in total bone length seems to be attained by six months of age in the os calcis and by six to eight months in the femur.

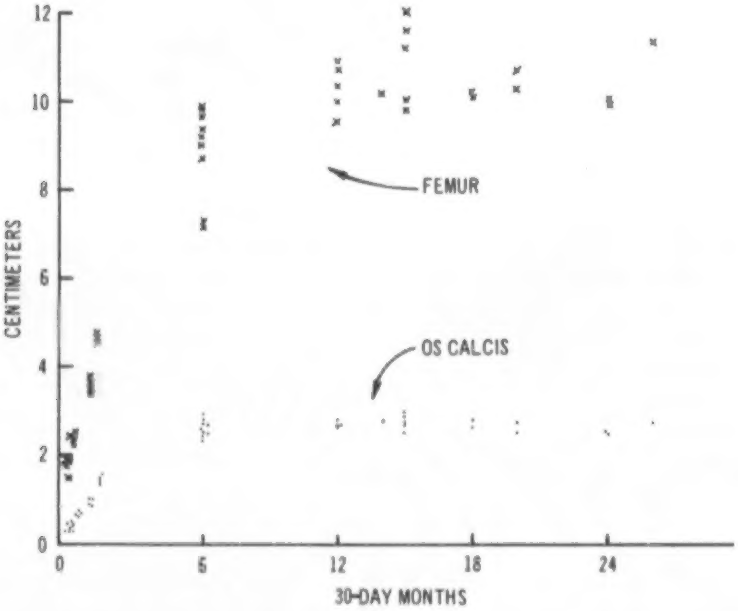

Figure 2. Total Bone Length at Various Ages.

The earliest appearance of epiphyseal closure in the distal femur and distal ulna has been observed at 12 months and in the distal humerus and proximal tibia at 15 months of age. Complete closure (Figure 3) in all samples obtained has been observed in the proximal os calcis by 12 months of age, in the distal ulna and distal femur by 15 months, and in the distal humerus and proximal tibia by 18 months. The epiphysis of the distal radius appears to remain open longer, but in samples obtained is closed at 20 months.

Since the study has not been completed and some of the age groups have only a few samples, data obtained thus far may be altered in accord with an increase in the number of samples.

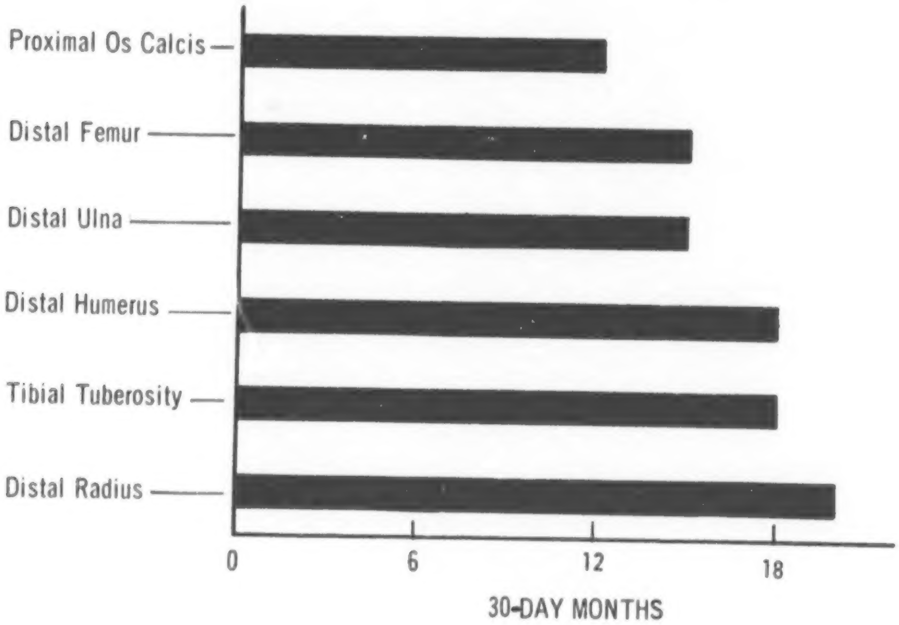

Figure 3. Ages of Observed Epiphyseal Closure.

Eruption of the Permanent Teeth of the Cat

E. Berman

As a criterion for determining physiological maturity in the cat, the mouths of 15 individuals in this colony were examined weekly to pinpoint the age at which their permanent teeth erupt. These observations are summarized in Figure 4. The data are expressed as the mean with 95% confidence limits.

The teeth in the upper jaw begin to erupt before those in the lower jaw. The anterior teeth (incisors) erupt first; then the eruption pattern proceeds posteriorly in an almost even pattern.

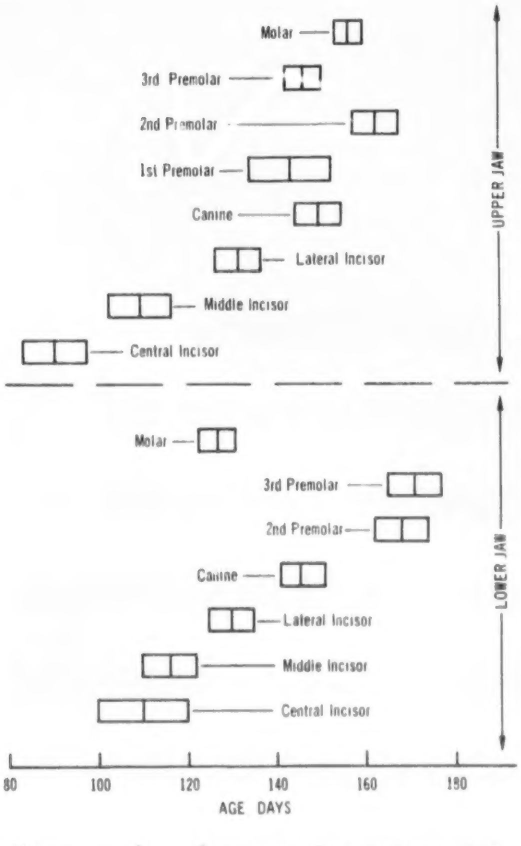

Figure 4. Age at Eruption of the Permanent Teeth of the cat (Mean ± 95% Confidence Limits)

Feline Embryonic Development

Neal S. Nelson and Joan Cooper

Embryo and placenta weights have been compiled for felines obtained by embryotomy at 2, 3, 4, 5, 6, 7, 8, or 9 weeks of gestation or collected at the time of birth. These values have been graphed to demonstrate the rate of embryonic growth. The values for the 0 and 1 week embryos shown in Figure 5 are based on published values of the size and volume of the feline ovum and 7-day morula.

The growth of both the embryo and placenta are extremely rapid in the first and second weeks of gestation. Growth of the placenta is exceptional since there is no placenta before nidation. After the 5th week of gestation the growth of the placenta almost stops, only doubling in weight from the 5th week to the end of gestation. The embryo continues to grow slowly, increasing in weight by a factor of 20 from the 5th week to the end of gestation.

Some embryos have been dissected so that organ and bone weight curves can be prepared, but analysis of the data is not yet complete.

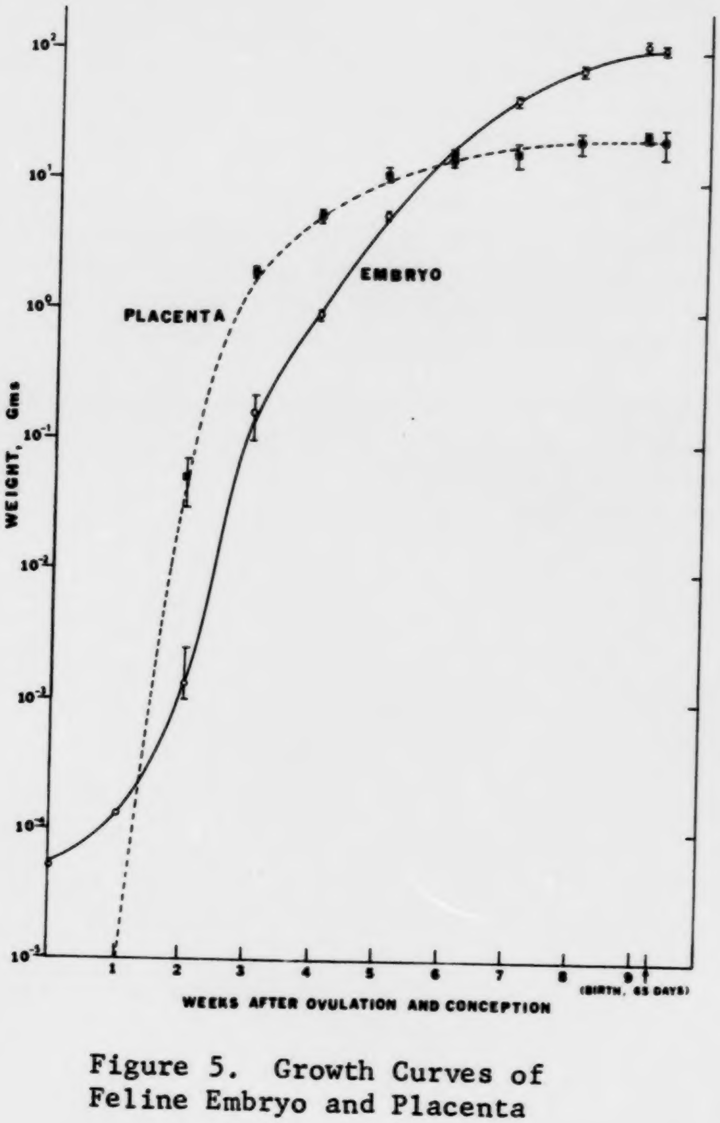

Figure 5. Growth Curves of Feline Embryo and Placenta

Response of the Peripheral Blood and Bone Marrow of the Cat to Severe, Acute Blood Loss

E. Berman and J. Wright

Four cats were severely and acutely bled to observe the response of the peripheral blood and the bone marrow to this stress.

The three males and one female, all between 8 and 18 months of age, were bled of a minimum of 1.9 to a maximum of 2.6 percent of their body weights at a single bleeding from the jugular vein.

The cats' blood was sampled on day 0, 1, 2, 3, 4, and 7 after bleeding. The bone marrow from one cat (1306) was aspirated from the proximal femur. The myeloid:erythroid ration of the bone marrow sample was 0.74:1 with an erythroid cell distribution of 3 percent rubriblasts, 7 percent promyelocytes, 21 percent rubricytes, and 20 percent metarubricytes. The magnitude of the response was less than expected for the rather severe stress imposed by the bleeding.

The reticulocyte count and the mean corpuscular volume showed uniform responses. In Figure 6, the absolute reticulocyte count in mm^3 of blood is shown to rise rapidly and definitely due to the stress of severe blood loss. A similar response is shown in Figure 7 depicting the change in mean corpuscular volume.

We had expected a more general reaction of the red cell components to this acute insult. Although an average of 1/3 of the blood volume was removed in a few minutes, no other circulating component reacted significantly.

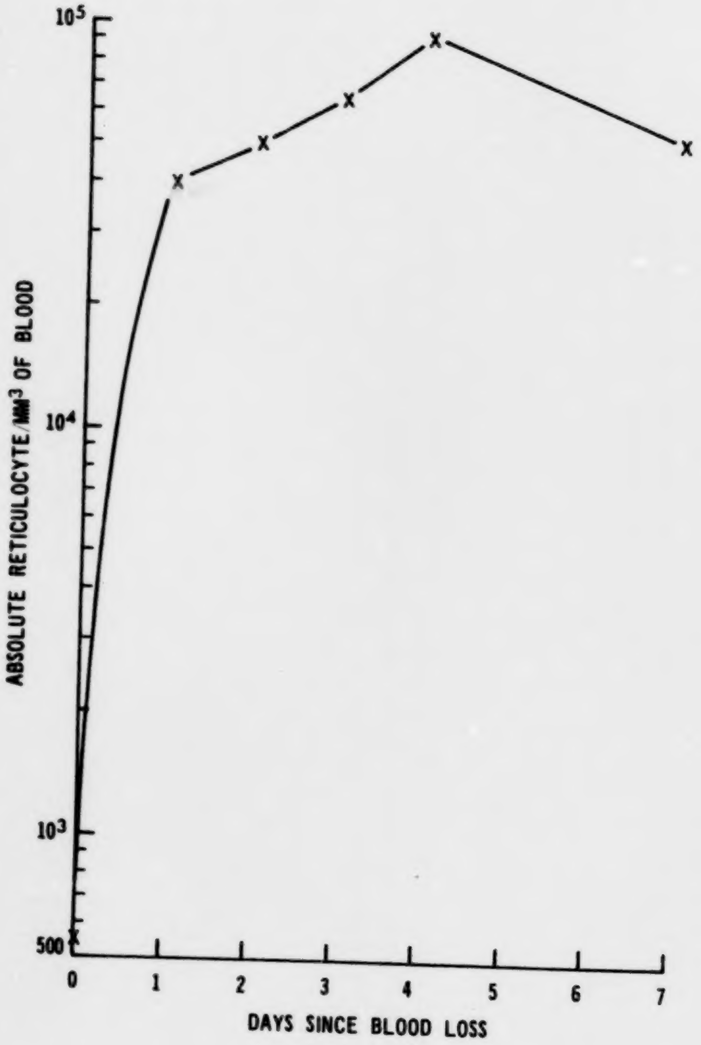

Figure 6. Absolute Reticulocyte Response to Severe Blood Loss in the Feline.

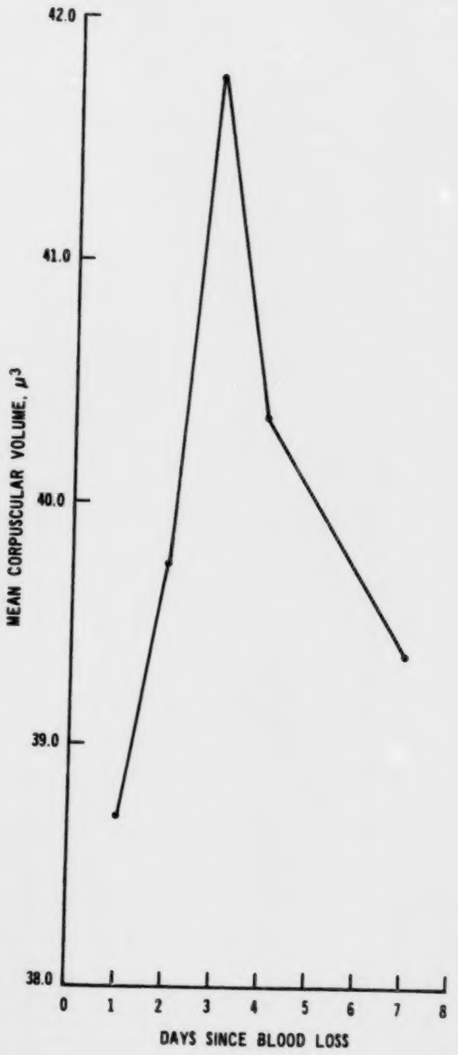

Figure 7. Mean Corpuscular Volume Response to Severe Blood Loss in the Feline.

Response of the Peripheral Blood of the Cat to Mild, Chronic Hemorrhage

E. Berman, B. Bolden

In using the cat in experimentation, we have frequently been required to sample the peripheral blood as a means for determining possible biological effects, especially those related to radiation exposure.

This study is being conducted to determine the response, if any, of the feline to various regimes of blood sampling.

Eight cats (five males and three females), all young to fully mature adults, were bled weekly, for 14 weeks, of 11.0 ml of jugular vein blood. Two days before this weekly stress was begun, during the weekly stress, and weekly for ten more weeks after the stress ended, the cats were given a complete blood count (CBC).

Figures 8-12 demonstrate some of the changes observed in the peripheral blood.

Figure 8 shows the depression of the hemoglobin through the stress period of 98 days. As soon as the periodic bleeding ended, the hemoglobin returned up to and above its previous normal value.

As shown in Figure 9, red blood cells decreased in response to the blood loss. During the recovery period, the red cell numbers increased to normal.

The micro-hematocrit (Figure 10) also decreased in response to the constant blood loss. Recovery was to its normal value. Figures 11 and 12 show the response curves of two Wintrobe Indices, Mean Corpuscular Hemoglobin (MCH) in $\mu\mu g$ (Figure 11) and Mean Corpuscular Volume (MCV) (Figure 12). Both decreased during the blood loss. Although recovery of the MCH began when the bleeding ended, the MCV began recovery before that time.

Although the number and quality of the red blood cells decreased because of the bleeding stress, they remained within normal limits for this colony.

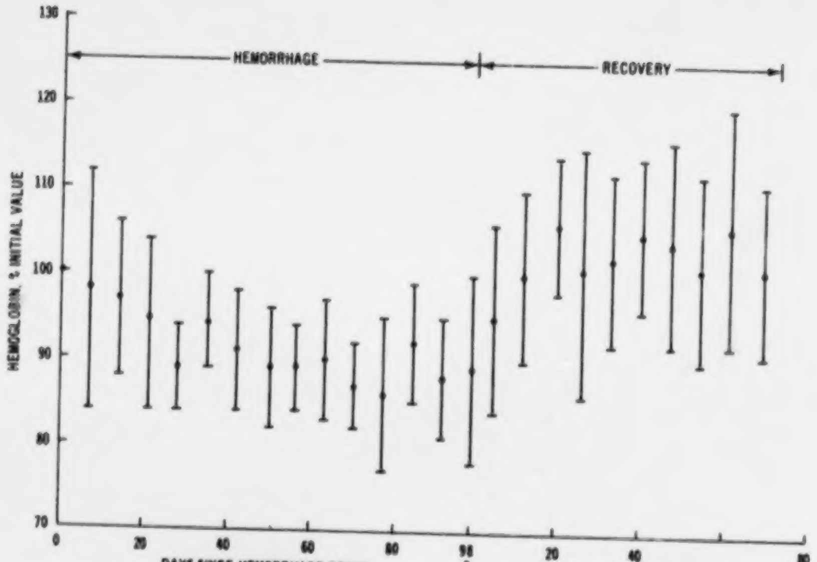

Figure 8. Response of Hemoglobin in Cats to Mild, Chronic Hemorrhage. Expressed as percent of initial value (day 0).

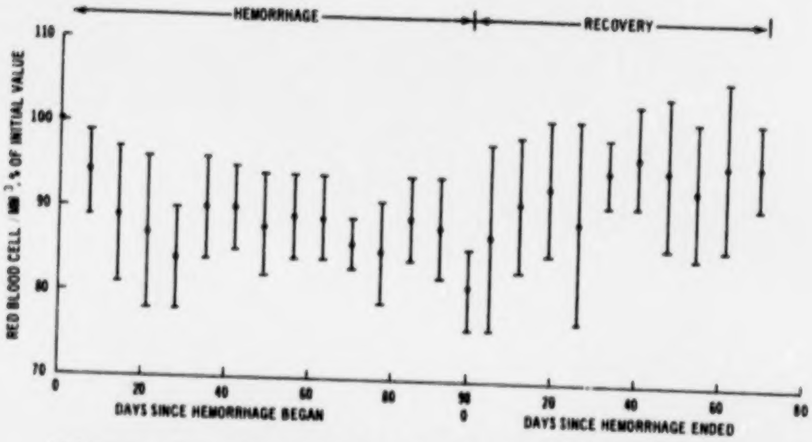

Figure 9. Response of Cat Red Blood Cell Numbers to Mild, Chronic Hemorrhage. Expressed as percent of initial value (day 0).

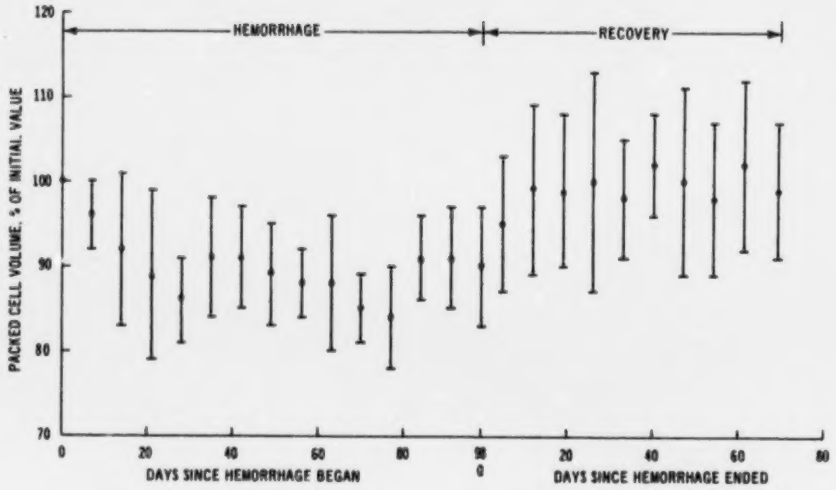

Figure 10. Packed Cell Volume (Microhematocrit) of the Cat in Response to Mild, Chronic Hemorrhage. Expressed as percent of initial value (day 0).

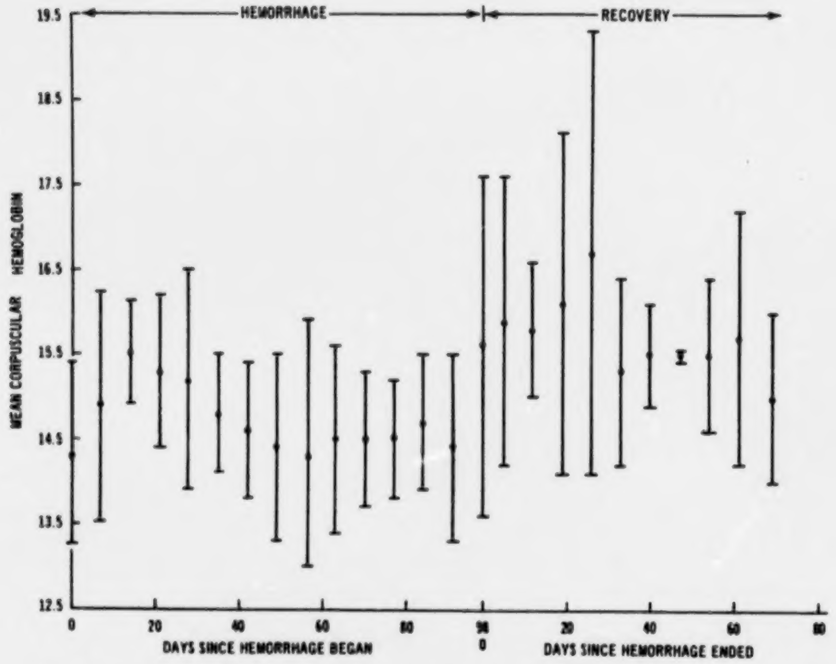

Figure 11. Mean Corpuscular Hemoglobin of the Cat in Response to Mild, Chronic Hemorrhage.

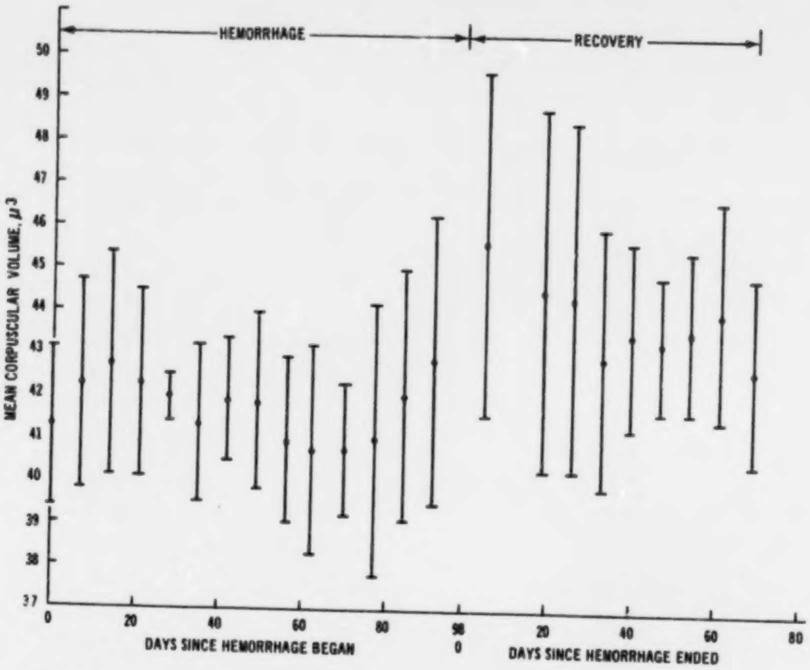

Figure 12. Mean Corpuscular Volume of the Cat in Response to Mild, Chronic Hemorrhage.

Mortality Rates

E. Berman, B. Ornduff, and D. Webb

Of prime importance in planning for the colony's use is the need for accurate death rates. With this information, we can schedule breeding, provide reserve stock for future use with some estimate of accuracy, and stipulate the number available at any age for long-term survival studies. Space limitations make planning especially critical; coupled with seasonal alterations, space limitations may cause crowding on the one hand to sparseness and lack of suitable subjects on the other hand.

Table 1 lists the age-specific mortality rates in this colony for various age groups. Of 452 cats included in this mortality table, 328 were born here. Experimental subjects, abortions and cesarotomies were not included. More than one-third (37%) of all full-term kittens born here have died before weaning (65 days of age). Twenty-two percent have died by the beginning of the 3rd day.

These data represent cats born or living in this colony between February 1969 and October 1970. No difference is presented in this table between data from 1969 and 1970, but it is our opinion that 1969 was a much more severe year regarding mortality than was 1970.

TABLE 1. AGE SPECIFIC MORTALITY RATES FOR THE TOXICOLOGIC STUDIES SECTION FELINE COLONY (2/69 - 10/70)

Life Period, Days	Number at Risk	Number Died	Percent Died
Stillborn	328	20	6.1
1	308	41	13.3
2	267	10	3.7
3	256	7	2.7
4	249	6	2.4
5	243	3	1.2
7	240	1	0.4
8	239	2	0.8
9	237	3	1.2
10	235	4	1.7
11	232	3	1.2
13	230	1	0.4
14	229	1	0.4
18	228	1	0.4
20	227	1	0.4
21-27	224	7	3.1
28-34	217	2	0.9
42-48	217	3	1.4
49-55	212	6	2.8
56-62	206	1	0.5
63-69	170	1	0.6
70-83	169	2	1.2
84-97	166	1	0.6
120-149	175	1	0.6
150-179	158	0	0.0
180-209	126	0	0.0
210-239	129	0	0.0
240-269	144	0	0.0
270-299	133	1	0.8
300-329	149	0	0.0
330-365	150	0	0.0
1 - 1 1/2 years	263	6	2.3
1 1/2 - 2 years	90	7	7.8
2 years	92	4 a	4.3

a. Died on Days 794, 872, 1014, 1052

Specific Diseases

E. Berman, C. Liddle, and J. Ward

Acute Necrotizing Hemorrhagic Cystitis

During late 1969 through 1970, six cases of acute necrotizing hemorrhagic cystitis occurred in mature male cats without evidence of urinary tract obstruction. The pathological changes produced were markedly similar in all cases and especially interesting in that these severe lesions were sharply demarcated at the vesico-urethral junction. Although it is not likely that obstruction or bacterial infection are concerned in this condition, the possibility of a viral agent producing or initiating this condition cannot be ruled out.

The following characteristics were common among these cases:

(1) Cystitis occurred in mature, breeding males, all previously normal.

(2) It followed an acute course, both in the appearance of symptoms and leading to death.

(3) Urolithiasis was absent.

(4) Pathological changes were essentially similar in all cases.

(5) Urethra was not, or was only slightly, affected.

A remarkable similarity was found in the gross pathological changes which were limited to the urinary bladders of all cases. The bladders were deep red or red-black in color with petechial and ecchymotic hemorrhages obvious over most of the surface of the organ. Usually there was only moderate distention with urine ranging in color from faint reddish yellow to dark red. In two cases reddish-brown amorphous jelly-like material resembling freshly clotted blood was found adhering to the bladder wall. Uroliths or other signs of obstruction were not present. The entire mucosal surface of the bladder was involved in the pathological process except for the region at the neck adjacent to the vesico-urethral junction which appeared unaffected (Figure 13). The line of demarcation between normal and pathological tissue was clearly delineated. A careful search was made from the bladder through the entire urethra for any occluding mass and none was found. In two cases there was evidence of damage to the urethral mucosa several centimeters distal to the bladder which was no doubt caused by catheterization.

Microscopically, hemorrhage was present in all layers of the bladder wall with marked necrosis and edema of the mucosa and submucosa. In two cases a slight tubular nephrosis was found and in one case a few petechial hemorrhages were found in a kidney along with one focus of intestinal nephritis. Microscopic examination in general substantiated the gross impression that the pathological process involved the bladder only.

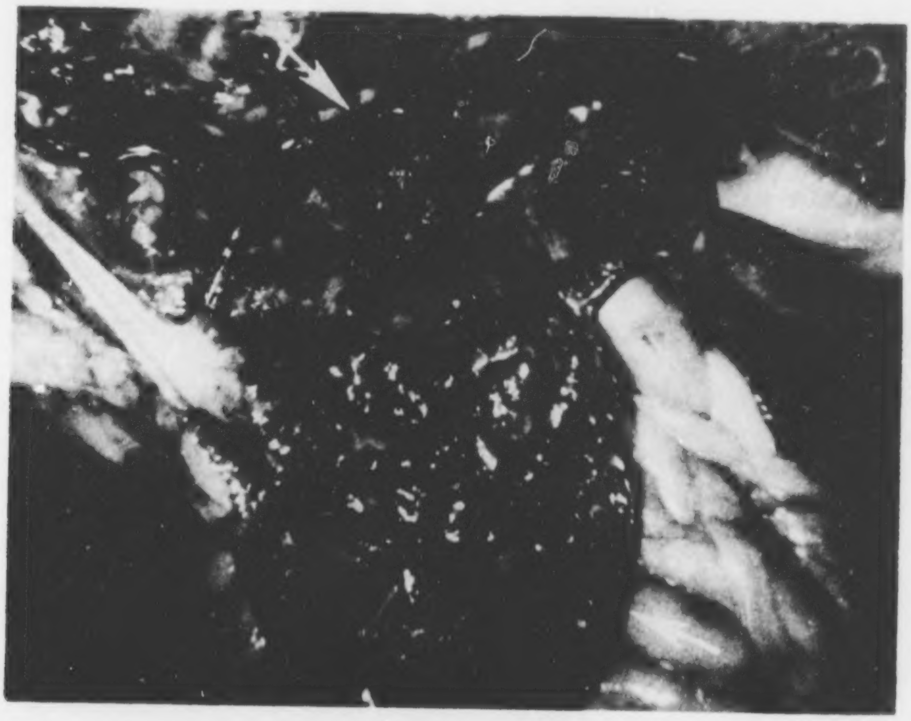

Figure 13. Acute Necrotizing Hemorrhagic Cystitis. Arrow points to edge of opened urinary bladder which is thickened, hemorrhagic, and necrotic. Note sharp delineation of normal appearing tissue at urethral junction.

Mild Chronic Cerebral Toxoplasmosis

Three mature male cats among many in the colony that have been sampled serologically by the indirect hemagglutination test for toxoplasmosis have exhibited increasing titers as shown in Table 2.

TABLE 2. INDIRECT HEMAGGLUTINATION TITER

Animal Number	1/8/69	2/16/70
1101	1:128	1:2048
1106	1:512	1:1024
1109	1:512	1:1024

These animals were clinically normal in all respects until they were killed and necropsied. In all cases, there were no gross abnormalities evident at necropsy which included examination of the central nervous system. Ophthalmoscopic examination of the eye grounds just prior to death was also normal.

Microscopically all three animals were found to have a mild chronic form of cerebral toxoplasmosis. Toxoplasma gondii cysts, perivascular lymphocytic cuffing and focal gliosis were found in the brains of all three cases (Figures 14, 15). In one case, a toxoplasma cyst was found in the myocardium but no organisms were found in other organs.

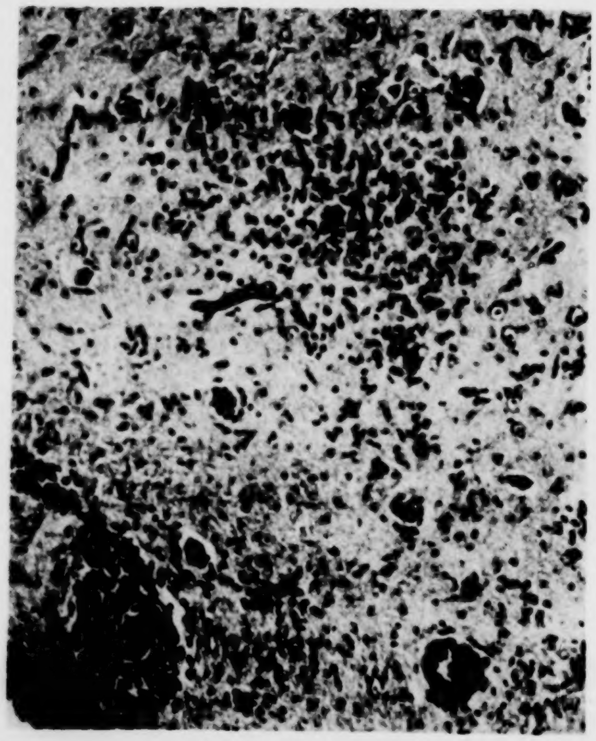

Figure 14. Toxoplasmosis. Perivascular cuffing and focal gliosis in the cerebral cortex

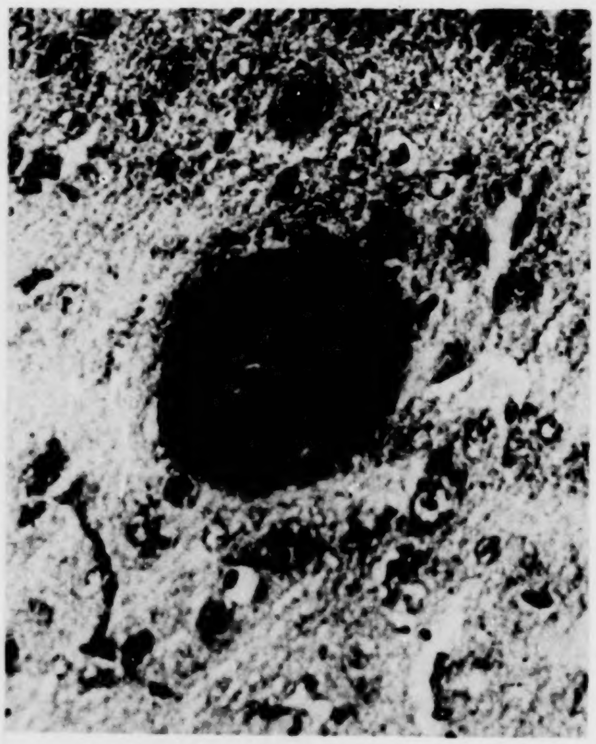

Figure 15. A Toxoplasma gondii Cyst Containing Numerous Organisms.

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METABOLIC STUDIES SECTION

Dr. DeWitt G. Hazzard, Chief

The Metabolic Studies Section develops knowledge of the immediate and delayed effects of radiation on metabolism and relates these effects to man. In order to determine the effects of radiation for evaluating health hazards and developing exposure standards, the section plans, conducts, and supports research which examines radiation-induced metabolic changes at the molecular, subcellular, cellular, and organ levels. Effects of irradiation are studied during the *in utero*, neonatal, juvenile, and adult stages of development and include both immediate and delayed responses.

In studies which have been pursued this past year, four source types of radiation emission have been examined: (1) x ray, (2) microwave, (3) ultrasound, and (4) radionuclide.

The competence of the Metabolic Studies Section may be divided into two major areas: (1) biological or biochemical and (2) physical chemical. Each of these research areas is amenable to testing radiation-induced changes in many regions of the frequency spectrum. Specific experimental projects are conducted in accordance with established priorities and exposure capabilities

A. ULTRAVIOLET SPECTRAL CHANGES IN BOVINE SERUM ALBUMIN AFTER IRRADIATION WITH MICROWAVES AT 2.45 GHz

John W. Allis and David E. Janes

At microwave frequencies and below, the interaction of electromagnetic fields with molecular systems is commonly studied by measuring the dielectric dispersion. Since a number of molecules of biological interest are dipolar, e.g., proteins, amino acids, and water, they have well-defined dispersion regions. Aqueous protein solutions have two principal dispersion regions as illustrated in Figure 1. These are due to the relaxation of protein molecules, in the range of 10^6 to 10^7 Hz, and the relaxation of water molecules, centered about the frequency 20 GHz (10^9Hz).

An additional dispersion between the two principal ones has been found in solutions of some proteins (1,2). It has been suggested that this dispersion (10^7 to 10^9 Hz) is due to segmental rotation of polar subgroups of the protein, or to water bound at the protein surface (1). This small dispersion encompasses the range of microwave frequencies between 10^8 and 10^9 Hz and is therefore important to the understanding of the interaction of radiation of this frequency with protein systems.

Small dipolar molecules such as amino acids and small peptides also have critical relaxation frequencies in this range.

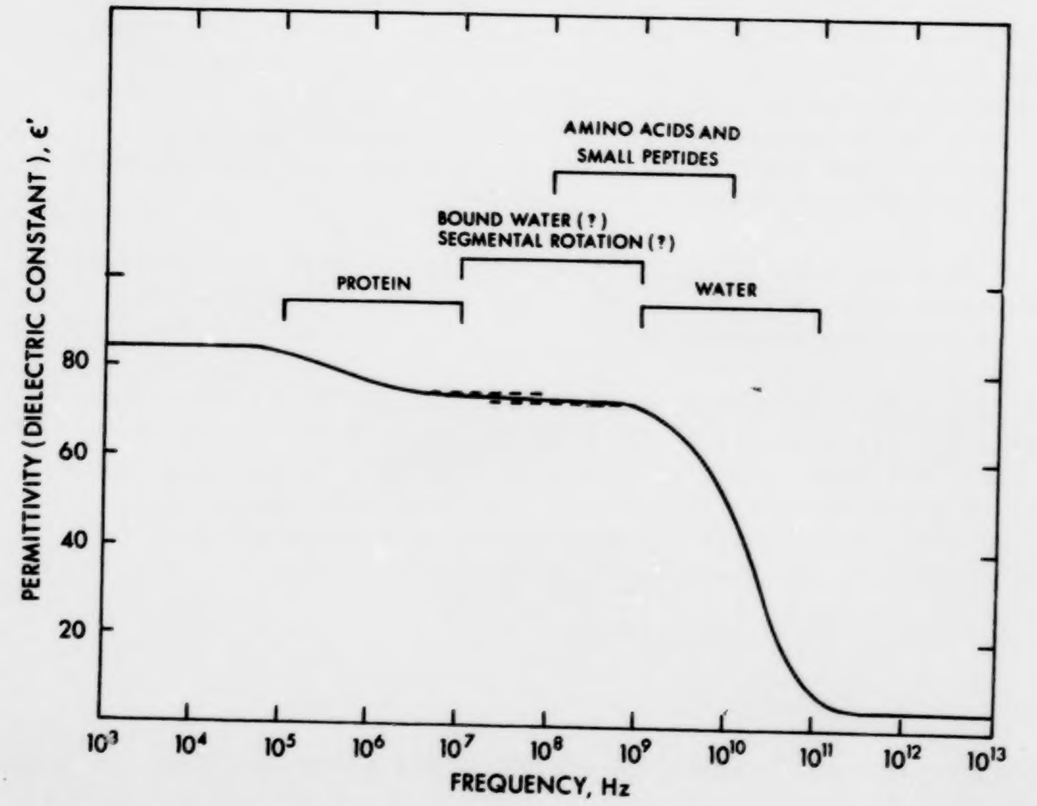

Figure 1. Dielectric Dispersion Curve for a Typical Protein in Aqueous Solution. Principal dispersion regions for the protein and for water are labelled. Dotted lines indicate limiting values of the principal dispersions, illustrating the presence of the additional dispersion.

The protein bovine serum albumin (BSA) is known to exist in more than one conformational state depending upon the pH. Below pH 4 the protein undergoes a reversible expansion first noted by Yang and Foster (3). It has also been demonstrated that in a narrow pH range near 4 the protein undergoes an isomerization before expansion takes place (4). This isomerized (F) form has been shown to be more flexible than the native (N) form (5). These conformational changes are summarized by the equation:

$$\begin{array}{ccccc} N & \rightleftharpoons & F & \rightleftharpoons & \text{Expanded} \\ pH > 4 & & pH = 4 & & pH < 4 \end{array}$$

This series of states offers the opportunity to compare the effect of microwave irradiation on forms of a protein with differing flexibilities, and therefore to evaluate at least one of the suggested mechanisms of interaction.

Summarized below are the results of experiments in which the N and F forms of BSA have been irradiated with microwaves at 2.45 GHz and compared to samples heated to an equivalent temperature in a water bath and to samples maintained at 25° C. The irradiated samples were heated in the water bath to 33° or 34° C, followed by irradiation at 25 or 30 $\mathrm{mW/cm^2}$ for 30 minutes. The temperature was monitored at 15-minute intervals and was always less than the water bath temperature. Duplicate samples were heated for the same length of time in the water bath and the remaining stock was maintained at 25° C.

The difference of the ultraviolet absorption spectra for treated vs. untreated samples has been measured from 215 to 310 nm. In this region of the spectrum, perturbations of the environment of tyrosyl, tryptophyl, and phenylalanyl residues contribute most of the spectral differences. BSA contains 21 tyrosyl groups of which about 7 are located at the protein surface in the native (N) form and an additional 3 to 5 are exposed in the F form. At low ionic strength most of the tyrosyls become exposed in the expanded state (6). The two tryptophyl and twenty-six phenylalanyl residues are more protected from solvent but can be perturbed under some conditions (7,8).

In Figure 2 the difference spectra of heated vs. untreated (H/U) and microwave irradiated vs. untreated (M/U) protein in the N form (pH 6) are shown. The H/U curve shows double peaks at 280 and 288 nm as well as a peak-trough combination near 230 nm. These changes are indicative of a tyrosine red shift (7,8). The M/U curve shows the same double peak at 280 and 288 nm, perhaps somewhat enhanced, an increase in $\Delta\epsilon_M$ near 260 and a broad band near 230 nm. Here the tyrosine red shift is accompanied by an additional effect that may be due to phenylalanine which absorbs near 260 and 215 nm. The red shift of tyrosine means some of the exposed residues are being removed from contact with solvent (9). This could be accomplished in several ways including aggregation.

The results of similar measurements taken on BSA mainly in the F form (pH 3.9) are shown in Figure 3. The H/U spectrum is nearly at the zero line indicating no spectral difference between the heated and untreated samples. The M/U curve (2 hours) is very similar to the corresponding curve of Figure 2. The only difference is the definite peak observed at 223 nm. The peak at 235 nm is probably the first part of the tyrosine red shift seen in Figure 2, but the trough has been overpowered by the peak at 224 nm. In this case, a further result has been found. After storage for 4 and 5 days at 3° C, the samples were rerun and the difference spectrum M/U was enhanced with more structure visible. In addition to the tyrosine peaks at 280 and 288 nm, peaks are evident at 259 and 265 nm. These are two of the five phenylalanine peaks. From these peaks it is not possible to tell if phenylalanine shift is blue or red, but if the peak at 223 nm is also from phenylalanine, it would indicate a red shift (9). (The reversal of the magnitudes of the spectral results for 2 hours and 4 days at wavelengths below 240 nm is not considered significant due to experimental difficulties in making the measurement in this region.) Again, red shifts indicate that residues in contact with solvent in the untreated protein are being removed from solvent contact after irradiation.

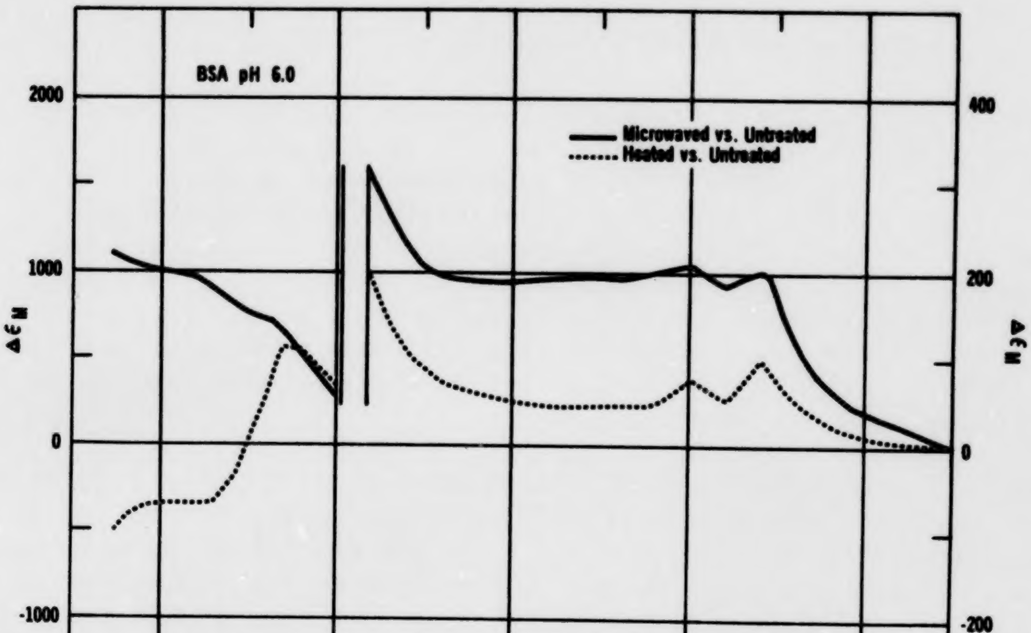

Figure 2. Difference Spectra of Microwave Irradiated and Heated BSA Measured Against Untreated Protein. Protein concentration is 0.250%, pH is 6.0, KCl is 0.01 M; Δϵ_M is the increment in molar extinction coefficient using a molecular weight of 69,000. Heated protein was held at 34° C for 45 minutes; irradiated protein was heated at 34° C for 15 minutes and exposed to 30 mW/cm^2 microwaves for 30 minutes, with solution temperature at 31.3° C.

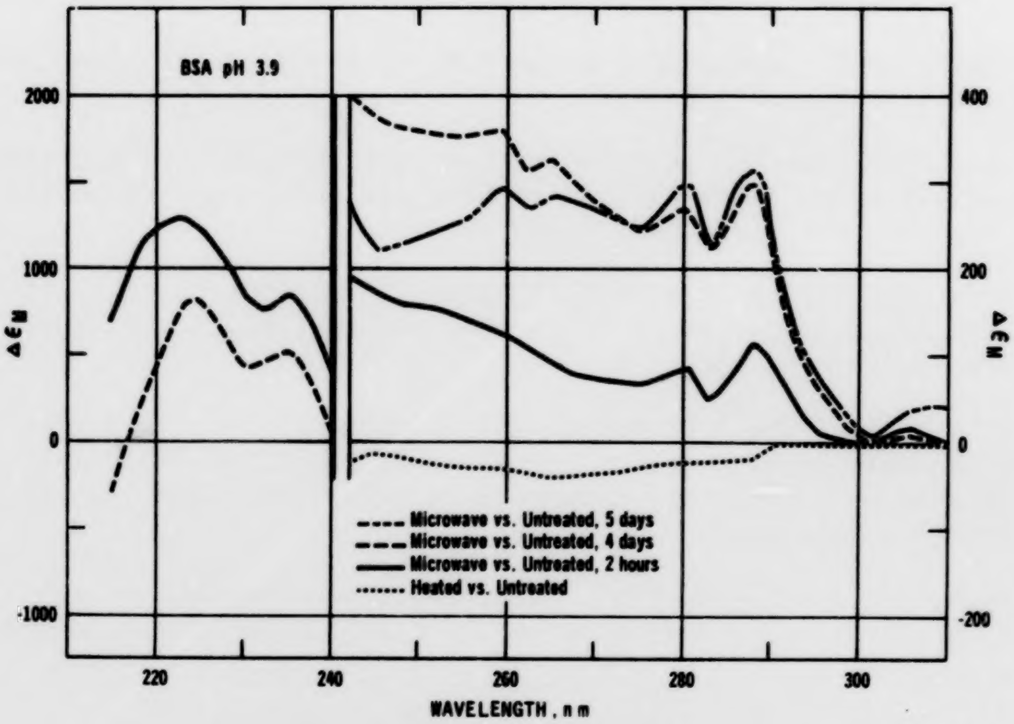

Figure 3. Difference Spectra of Microwave Irradiated and Heated BSA Measured Against Untreated Protein. Protein concentration is 0.230%, pH is 3.9, KCl-HCl is 0.02 M; $\Delta\epsilon_M$ is the increment in molar exinction coefficient using a molecular weight of 69,000. Heated protein was held at 33° C for 45 minutes; irradiated protein was heated at 33° C for 15 minutes and exposed to 25 mW/cm^2 microwaves for 30 minutes, with solution temperature at 31.5° C.

In summary, early experiments have shown some spectral differences between microwave irradiated BSA and heat treated protein when the protein is in both N and F forms. In the N form the changes appear to involve tyrosine and perhaps phenylalanine and the changes appear to be complete within two hours after irradiation. For BSA in the F form the changes almost certainly involve both tyrosine and phenylalanine; the changes are not complete within 2 hours of irradiation but are complete in 4 days.

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B. SOME PRELIMINARY OBSERVATIONS ON AUTOIMMUNE RESPONSE IN RATS EXPOSED TO 2450 MHz MICROWAVES

Gopal P. Kamat

Thermal injury in patients and experimental animals may be associated with a variety of immunological abnormalities. Prolonged survival times of skin allografts in burned patients (1), associated with a diminution of their ability to exhibit delayed hypersensitivity reactions (2), formation of anti-skin antibodies in burned men, horses, dogs, rabbits, rats and mice (3-8) antibodies to autologous erythrocytes in burned rats (9, 10) and "heterophile" antibodies against rat erythrocytes and human gamma globulin in guinea pigs and rabbits and in some patients with severe skin burns (11) have been observed.

The objective of this investigation was to examine the possible autoimmune response and pathological changes in rats (cumulative biological effects) exposed to whole-body radiation with 2450 MHz microwaves. The experimental animals were exposed to acute sublethal, repeated doses of 2450 MHz microwaves and tested for the appearance of organ specific antibodies and histopathological changes in organs. Although organ specific autoantibodies and histopathological changes were not observed, the results will be described and their pertinence compared with the results obtained by thermal injury.

Materials and Methods

Experimental Animals and Method of Microwave Radiation Injury

Albino rats, weighing 125-150 g were used for microwave exposure. The rat was placed in a perforated plastic cage, 100 cm in front of the microwave generator horn on the main beam axis with its side facing the radiation source and irradiated several times with 2450 MHz microwaves. The average power density was 100 ± 15% mW/cm^2. The experimental conditions were as described on p. 147. As shown in Table 1, five rats (group I) received two exposures of 8 minutes each and then two exposures of 10 minutes each, one exposure per week for 4 weeks. Five other rats (group II) received three exposures of 15 minutes each and then four exposures of 13 minutes each on day 3, 6, 8, 10, 22, 24 and 29 of the month. The third group of five rats was sham-irradiated and served as a control. The rise in the rectal temperature of the 3 groups of rats was read to ± 0.1°C with a nylon coated thermistor probe, time constant 3 seconds, immediatedly after exposure. One month after the first exposure, the animals were sacrificed and serum samples were obtained from each animal by cardiac puncture. All serum samples were stored at -20° until used.

The organs (testis, eye lens, liver, spleen, lung, heart, kidney, thyroid and brain) were freed of all surrounding connective tissue and minced with scissors. A 1.0 percent (w/v) homogenate was prepared in

0.85 M saline solution and stored at -20°C until used. Double diffusion gel precipitation was performed according to the method of Ouchterlony (12). The tissue samples were fixed in a 10 percent formalin solution; sections were stained with hematoxylin and eosin for histological study.

TABLE 1. MEAN RECTAL TEMPERATURE AFTER MICROWAVE EXPOSURE

No. of Animals	Date of Irradiation	Minutes of Irradiation	Rectal Temp., °C		Temp. Rise, °C
			Mean	Std. Error	
			Group I		
5	2/25/70	8	40.70x[b]	.25	3.05
	3/ 4/70	8	40.98x	.09	2.99
	3/11/70	10	42.16y	.19	4.02
	3/18/70	10	41.58z	.12	3.47
			Group II		
5	4/ 3/70	15	42.37	.50	4.77
	4/ 6/70[a]				
	4/ 8/70	15	43.32[c]	.18	5.72
	4/10/70	13	42.58	.22	4.77
	4/22/70	13	43.27	.29	5.62
	4/24/70	13	42.73	.36	4.88
	4/29/70	13	42.83	.07	5.73

a. Data for this date was lost due to malfunction of equipment.

b. Means with the same letters are not significantly different; those with different letters are significantly different ($P \leq .05$).

c. Two rats died after this exposure, leaving only three animals for this and subsequent measurements.

Results

Body Weight and Rectal Temperature Before Exposure

All three groups of rats grew at approximately the same rate; no statistically significant effect of radiation treatment was detected. No statistically significant differences in mean rectal temperature before exposure were found among treatment groups or among dates of exposure.

Rectal Temperature After Irradiation

Mean rectal temperatures taken immediately after microwave exposure are given in Table 1. Separate analyses of differences by date of irradiation within each treatment group showed no statistically significant differences within group II. Within group I, however, rats irradiated on 3/11/70 had the highest mean temperature and those irradiated on 3/18/70 had intermediate temperature; there was no statistically significant difference between the two groups having the lowest temperature. Group II had a significantly higher mean temperature than group I.

Thyroid and Eye Lens Weights

Mean thyroid and eye lens weights were determined for group I and control rats. Mean thyroid weights of group I rats (0.13 gram) were significantly greater ($P \leq 0.01$) than those of control rats (0.016 gram). Mean eye lens weights of 0.07 and 0.10 gram for the control and group I rats were not significantly different.

Double Diffusion Technique

The homogenates of testis, eye lens, liver, spleen, lung, heart, kidney and brain of each of the rats of the control group and treated group I and group II were tested with their own serum for the presence of the precipitin line. None of the sera from the control group or the treated groups gave a positive reaction with any of the organ extracts. The results showed that the serum antibody could not be detected by double diffusion technique in microwave irradiated rats of either group.

Histopathological Sequelae of Microwave Radiation

The organs (testis, eye lens, liver, spleen, lung, kidney, heart, thyroid and brain) of the microwave irradiated rats from group I and group II and of the control rats were examined for histopathological changes. The only significant observation in the microwave irradiated group II rats was the atrophy of the tubules of the testis. No other significant histopathological changes were observed in any of the microwave irradiated rats. A mild to moderate murine pneumonia was observed both in the microwave irradiated and nonirradiated rats.

Discussion

The appearance of auto and heteroantibodies after thermal injury may be a consequence of the release of intracellular antigens and/or alteration of autologous antigens (13). In this investigation, however, no serum antibody against any of the 8 organ antigens of the microwave irradiated rats could be detected by the immunodiffusion technique. The absence of the organ-specific autoantibodies supports the suggestion that there was probably no release or very insignificant release of intracellular antigens and/or alteration of autologous antigens in the microwave irradiated rats.

Rapaport et al. (14) reported the formation of antibodies against testicular antigens in a high proportion of guinea pigs (68 percent) exposed to thermal injury of the testis (average temperature at the burn site: 220°C). Testicular injury caused by the unheated electrode puncturing the entire thickness of the testis resulted also in the production of testis antibody in a smaller number of animals (15 percent). The results of Rapaport et al. suggest that, under certain conditions, thermal injury may set in motion an autoimmune process leading to organ damage (14). Why no such response was seen in this study, especially since there was testicular damage, is unclear. All the rats died when the whole-body microwave irradiation exposure resulted in the mean rectal temperature rise of about 5.73°C. Although the cumulative biological effects of the microwave radiation obtained in the experimental animals may or may not be the same as those observed in humans, they might be useful in evaluating the effects of microwave radiation in man.

In conclusion, the whole-body 2450 MHz irradiation of rats did not elicit the formation of organ-specific antibodies under the conditions of this study.

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C. ABSENCE OF IMMUNOGLOBULIN AGGREGATES IN HUMAN PLASMA WARMED WITH 2450 MHz MICROWAVES

Gopal P. Kamat

Introduction

Restall et al. (1) described a microwave blood warmer capable of warming a bag of human blood (450 ml blood and 67.5 ml acid citrate dextrose) from 5°C to 35°C in approximately 1 minute. The blood warmer utilized a magnetron tube with an oscillating frequency of 2450 MHz and a power output of 1,000 watts to produce microwaves. Restall et al. (2) also reported that such warming did not damage human blood. The results led the authors to believe that the microwave blood warmer was a clinically useful piece of equipment.

Kamat and Janes (3) reported the formation of human immunoglobulin G (IgG) aggregates but no qualitative gross changes in the structure of the main soluble IgG fraction when human IgG was irradiated in vitro with 2450 MHz microwaves (temperature 50°C for 40 minutes). Kamat and Tell (4) have also observed changes in the immunological and physico-chemical properties of 2450 MHz microwave-irradiated human IgG (temperature 50°C for 40 minutes). Microwave irradiated human IgG provoked skin-sensitizing reactions in guinea pigs; the antigen-antibody precipitin properties of the microwave irradiated human IgG were different from those of the native human IgG, and more disulphide bonds were available for reduction with 2-mercaptoethanol in the irradiated antigen than in the native antigen. The IgG aggregates have a number of biological activities that are not demonstrable in pure preparations of native 7S IgG. The aggregated IgG binds rheumatoid factor (5), can provoke skin-sensitizing reactions in guinea pigs (6), fixes complement (7), and causes erythema-wheal reactions in humans (6). It is possible that IgG altered non-specifically by heating could be responsible not only for the presence of circulating autoantibodies but also for the induction of many allergic phenomena.

In view of these changes in the immunological and physico-chemical properties of the microwave-irradiated human IgG and of the IgG aggregates produced by heat, a study was undertaken to isolate and characterize the formation of immunoglobulin aggregates in human plasma irradiated with a 2450 MHz microwave blood warmer.

Materials and Methods

Microwave irradiated and non-microwave irradiated acid citrate dextrose (ACD) human plasma was supplied by the Mayo Clinic blood bank, Rochester, Minnesota. ACD human plasma was taken from 500 ml units of donor blood of various storage ages (up to 21 days).

The antiserum was prepared in rabbits against human gamma globulin (Cohn Fraction II) maintained at 50°C for 30 minutes by the radiation from a cycling microwave generator operating at a frequency of 2450 MHz. The actual irradiation time was 9 minutes. Equal volumes of Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) and of approximately 1.0 g% antigen solution in 0.85% NaCl were emulsified together to give a water-in-oil emulsion. Rabbits were immunized by injecting 1.0 ml emulsion intramuscularly at several sites twice per week for one month. Seven days after the last injection, the rabbits were test bled (about 10.0 ml) from a marginal ear vein, the serum was separated, and the antibody titer was tested by the precipitation test (8).

Human blood (450 ml) and acid citrate dextrose (67.5 ml) was warmed in a microwave blood warmer (Holaday Industries Inc., Hopkins, Minnesota) from 5°C to 35°C in approximately 1 minute (1). The temperature of the blood increased in the microwave blood warmer at approximately 0.5°C per second. The field configuration was that of a shaped electrical field. The total power applied to the entire unit of blood was 1,000 watts at 2450 MHz. The blood bag was placed in a plastic cylinder shielded with metal at both ends to protect the blood from overheating at the tapered ends of the plastic bag. It was rotated on its long axis at 20 rpm while being exposed to the microwaves.

Microwave irradiated and nonirradiated human plasma samples were centrifuged at 103,000 g at 4°C for 90 minutes using a Spinco rotor 40.3 in a Beckman model G ultracentrifuge. The supernatant was carefully withdrawn with a Pasteur pipette. The pellet was resuspended in 0.1 M barbitone buffer which was acidified with HCl to pH 5.00.

Immunoelectrophoretic analysis was performed by the method of Grabar and Williams (9) using 0.1 M barbitone buffer, pH 8.2.

Results

The pellet and the supernatant from the microwave irradiated and nonirradiated human plasma and the normal human gamma globulin (Cohn Fraction II) were tested by immunoelectrophoresis using antiserum against microwave irradiated human gamma globulin (Cohn Fraction II). The results are shown in Figure 1. The microwave irradiated human plasma contained a larger quantity of the pellet and lipid layer than the nonirradiated human plasma. There was no significant difference between the electrophoretic mobility of the main precipitin lines (Possibly IgG) obtained from the pellets of the microwave irradiated and nonirradiated human plasma and that of the main precipitin line obtained from normal human gamma globulin (Cohn Fraction II). The same observation was true for the electrophoretic mobility of the main precipitin lines (possibly IgG) obtained from the supernatant from microwave irradiated and nonirradiated human plasma. No significant difference in the electrophoretic mobility of the faint precipitin lines (possibly IgM, IgA, IgE, and IgD) obtained from the pellet and the supernatant of the microwave irradiated and nonirradiated human plasma was observed. There was no "splitting" of the precipitin lines and no new antigens appeared in the human plasma warmed from 5° to 35°C with 2450 MHz microwaves in about 1 minute.

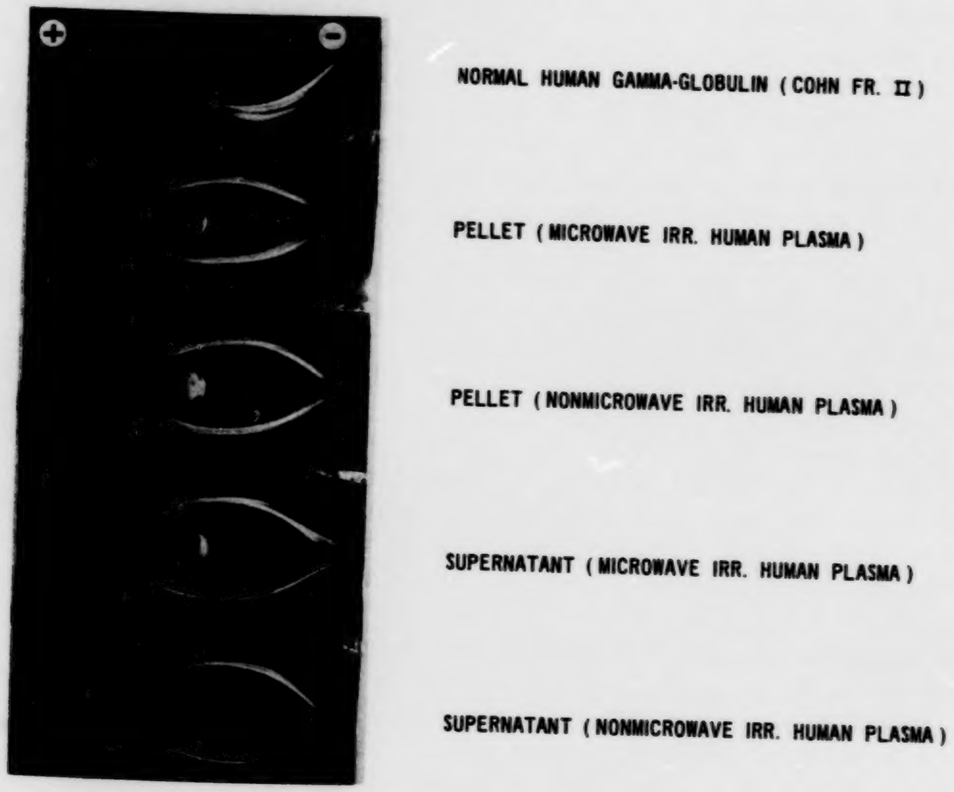
+
−
NORMAL HUMAN GAMMA-GLOBULIN (COHN FR. II)
PELLET (MICROWAVE IRR. HUMAN PLASMA)
PELLET (NONMICROWAVE IRR. HUMAN PLASMA)
SUPERNATANT (MICROWAVE IRR. HUMAN PLASMA)
SUPERNATANT (NONMICROWAVE IRR. HUMAN PLASMA)

Figure 1. Immunoelectrophoretic Pattern of Microwave and Nonmicrowave Irradiated Human Plasma. The human plasma was microwave irradiated as described in Materials and Methods. Antiserum: Rabbit anti-microwave irradiated Cohn fraction II. The pellet was isolated by centrifuging the irradiated and nonirradiated human plasma at 103,000 g at 4° C for 90 minutes in Spinco rotor 40.3.

Discussion

The stability of the immunoglobulins is a critical requirement both to experimental immunologists and to clinicians responsible for the prophylactic or therapeutic administration of human blood or antibody preparations. Hence, structural changes occurring in the immunoglobulin molecule (mainly 7S IgG) following storage or after warming are of great interest. The structural changes in the 7S IgG molecule could result in alterations in antigenicity which could conceivably be associated with deleterious immunological responses or to some relative ineffectiveness due to accelerated removal from the circulation (10).

Presumably untoward reactions are usually avoided by the practice of administering crude human 7S IgG preparations, which possess other serum proteins capable of stabilizing the immunoglobulin molecule.

The results of this study show the absence of the significant immunoglobulin aggregates detectable by immunoelectrophoresis in the human plasma warmed with 2450 MHz microwaves from 4-6°C to 35°C in about one minute. The absence of the detectable free SH groups, and no change in the rotary dispersion constants of the native human IgG and IgG heated for ten minutes at 37°C (11), are in support of our results. No significant change was observed in the rheumatoid factor reactivity and minimum skin reactive dose. Human complement fixation was absent in the native human IgG and IgG heated for 10 minutes at 18-20°C and at 37°C (11).

The results of this investigation suggest that human blood warmed with 2450 MHz microwaves from 4-6°C to 35°C in about 1 minute and used for the prophylactic or therapeutic administration in humans perhaps may not be associated with deleterious immunological responses.

Conclusions

(1) There was an absence of the significant immunoglobulin aggregates (detectable by immunoelectrophoresis) in the human plasma warmed with 2450 MHz microwaves from 4-6°C to 35°C in about 1 minute.

(2) No changes (e.g., "splitting") in the immunoelectrophoretic precipitin line were demonstrable in the warmed human plasma.

(3) The absence of the structural changes and presumably the absence of alterations in antigenicity of immunoglobulins suggest that the prophylactic or therapeutic administration of human blood warmed with 2450 MHz microwaves from 4-6°C to 35°C in about 1 minute perhaps may not be associated with deleterious immunological responses.

Acknowledgment

The author wishes to thank Mrs. Mary K. Howes, Bureau of Radiological Health, for her excellent technical assistance. I am grateful to Dr. H.F. Taswell, Medical Director, Blood Bank, Mayo Clinic, Rochester, Minnesota for kindly sending me samples of microwave irradiated and nonirradiated human plasma.

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D. ENZYME INACTIVATION IN VITRO WITH 2450 MHz MICROWAVES

Gopal P. Kamat and John W. Laskey

Investigations on enzymes irradiated in vitro in electromagnetic fields of various frequency ranges have shown changes in enzyme activity (1-4). These results were obtained under a variety of conditions; however, there was indication that temperature rise might be a dominant factor (4).

The object of this study was to determine if the thermoresistant bacterial alpha-amylase (Bacillus subtilis) could be inactivated with 2450 MHz microwaves at experimental conditions which maintained the temperature below the thermocritical temperature for this enzyme.

This enzyme was selected because it is available in a pure form, a large amount of information is available concerning it, and it is thermostable, which distinguishes it from alpha-amylases of other origins.

Microwave Irradiations

Irradiations were performed at 2450 MHz from a microwave generator equipped with a 10 dB, standard-gain, pyramidal horn (Model PPS-25AS, Varian Industrial Microwave Systems, San Carlos, California). The enzyme samples were exposed in an anechoic chamber approximately 10 x 20 x 8 feet built with Emerson and Cumings CVB-24 anechoic material and with a performance of -36 dB ± 2 dB average reflectivity. The enzyme samples (10 ml) in polystyrene tubes were placed 100 cm in front of the transmitting horn on the main beam axis. The average power density measured along the principal axis by a tuned dipole was 100 mW/cm^2 ± 15 percent. The power density fell by 5 percent when measurements were performed 10 cm off the axis. The detecting element, a PRD type N 685-2 thermoelectric power head was connected to a PRD type 6685 thermoelectric power meter. A cable (RG-8/u) having an attenuation of -6.5 dB at 2450 MHz connected the dipole antenna to the detector. The average temperature of the enzyme samples was read with an ice-bath referenced chromal-alumel thermocouple. The temperature measurements were taken immediately after the microwave generator was turned off.

Determination of Alpha-Amylase Activity

The alpha-amylase activity was determined using the method of Fischer and Stein (5) with slight modifications in the starch concentration and alpha-amylase pH. A starch solution of 1.0014% was incubated with alpha-amylase at pH 5.9, followed by the absorbance measurement of reducing sugars at 540 mμ with alkaline sodium 3,5-dinitrosalicylate.

The unit of the enzyme is that amount of enzyme which in 3 minutes liberates from starch, reducing substances equivalent to 1 mg of maltose hydrate (about 1 μg of crystalline enzyme). The unit of alpha-amylase was obtained from the following equation:

$$\frac{\text{A540 Exp.}}{0.465} = \text{units of alpha-amylase}$$

where A540 Exp. represents the experimental value of the absorbance at 540 mμ. One mg of maltose hydrate after reduction with 3,5-dintrosalicylic acid has an absorbance of 0.465 at 540 mμ.

Enzyme Activities of Bacterial Alpha-Amylase

Eight samples (10 ml each) of bacterial alpha-amylase solutions were microwave irradiated under different experimental conditions. Four samples (Samples A,B,C and D) were microwave irradiated at 106.8 mW/cm^2 with a total irradiation time of 15 minutes. These four samples were cooled during irradiation with nitrogen cooled in a dry ice-acetone mixture so that the temperature of these solutions did not rise above room temperature (21°C). The temperature was measured at two points: The top of the solution and the bottom of the solution with two thermocouples attached to the tube containing the solution (Figure 1). The time and temperature curves for each of the four samples are shown in Figure 2. The results show that the temperature of the four samples was in the range of 16° to 20°C and that the temperature at the top of the solution was higher than that at the bottom of the solution, the maximum difference being about 2°C.

Samples E and F were microwave irradiated at 106.8 mW/cm^2 for a total irradiation time of 15 minutes but the samples were not cooled with cold nitrogen during irradiation. The time and temperature profiles of these samples are shown in Figure 3. Sample E attained a maximum temperature of 31.7°C and sample F attained a maximum temperature of 38.05°C. The temperature difference between the top and the bottom of the solution was about 1°C.

NOT REPRODUCIBLE

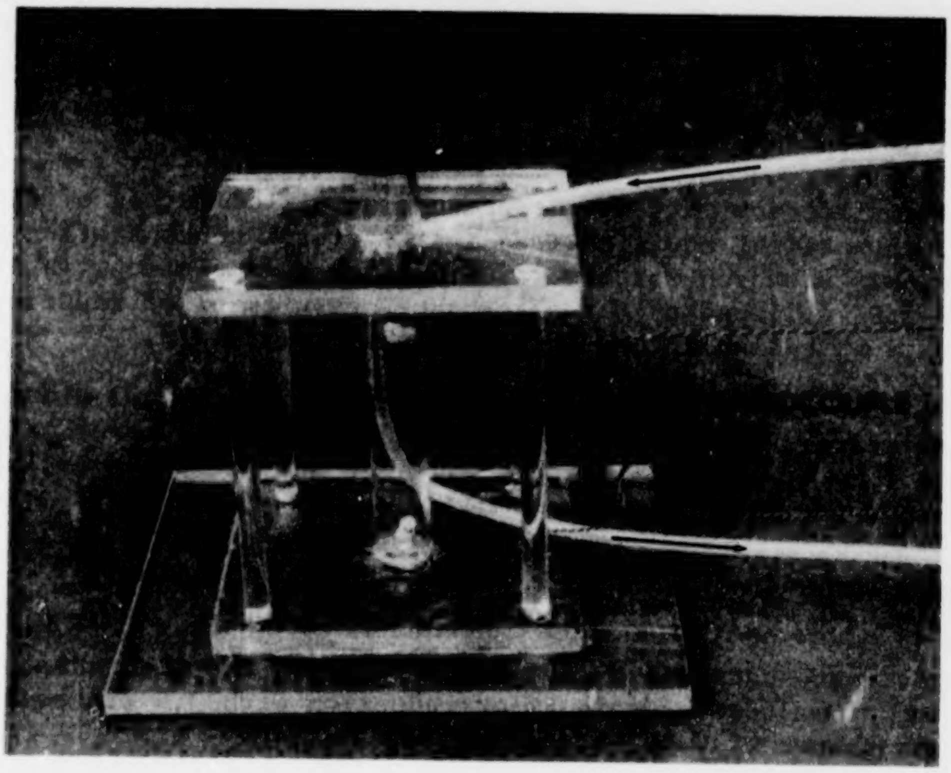

Figure 1. Apparatus Used to Expose 10-ml Samples of Enzyme to Microwaves. Screw-cap tube was 12.4 cm long, with a 1.4 cm inside diameter and 1.7 outside diameter. Cold nitrogen tubes penetrated exposure tube 2 and 9 cm from bottom. Thermocouple probes were mounted 1.5 and 7.0 cm from bottom of tube.

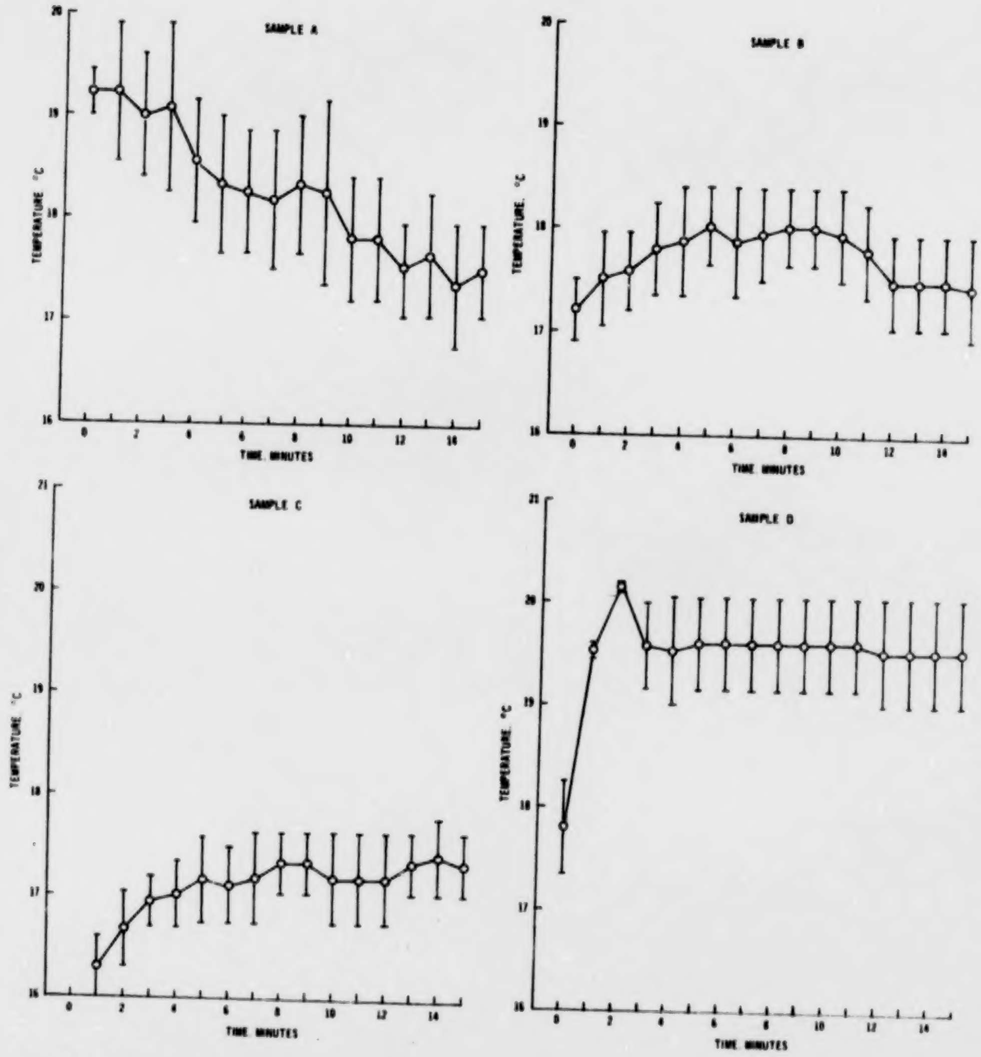

Figure 2. Temperature of Enzyme During Microwave Exposure. Extremes of vertical lines represent temperature at top and bottom of tube. Mean temperatures are represented by curve.

Samples G and H were irradiated at a power density of 280 mW/cm² until the temperature of the solutions was about 65°C in 10.5 minutes and then the solutions were maintained at 63.7 ± 11.1°C for 15 one-minute exposures separated by about 20 seconds. The average power density during the 15 one-minute exposures was 207.5 mW/cm². The time and temperature profile for samples G and H are shown in Figure 3. The maximum temperature attained by sample G was 69.25°C and by sample H was 74.8°C. The temperature difference between the top and the bottom of the solution was 12.75°C for sample G and 22.2°C for sample H. Two samples, I and J, were heated in a water bath at about 75°C for 15 minutes. The time and temperature profiles of these samples are shown in Figure 4. Samples K and L were untreated enzyme samples and served as controls.

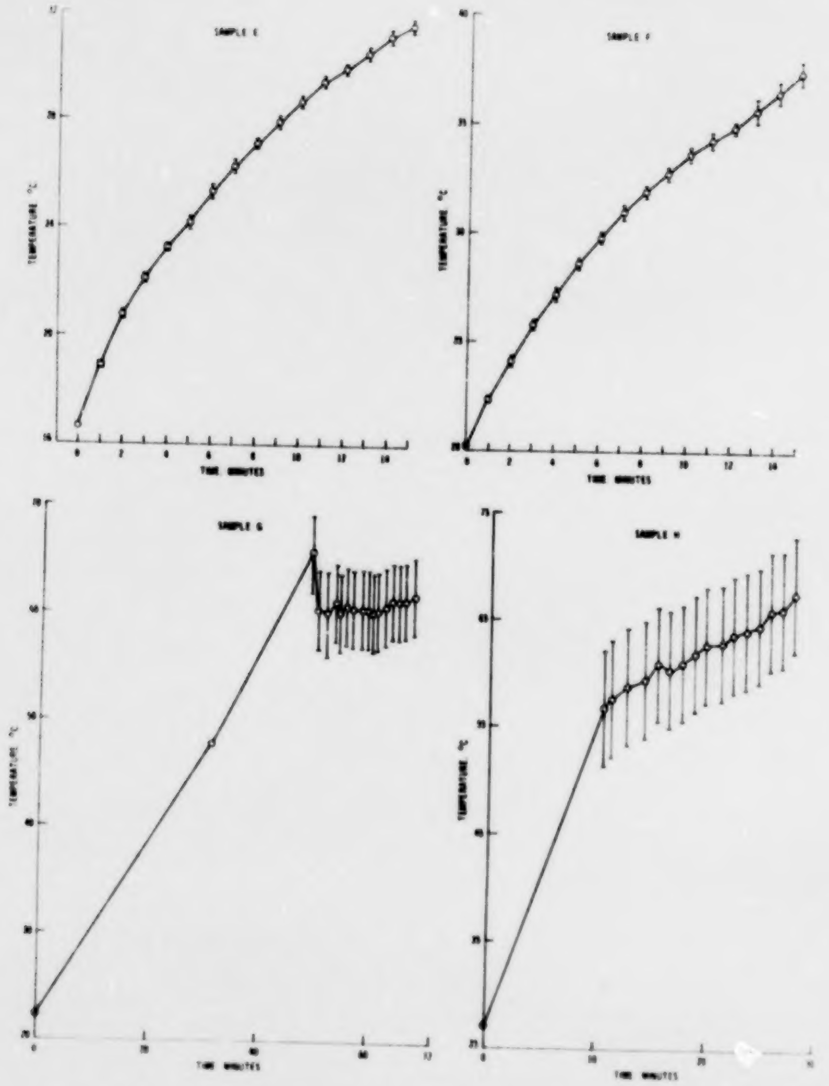

Figure 3. Temperatures of Enzyme Sample During Microwave Exposure. Extremes of vertical lines represent temperature at top and bottom of tube. Mean temperatures are represented by curve.

The activity of the bacterial alpha-amylase was determined for five aliquots of 250 μg each for each of the twelve samples. The results given in Table 1 show that uncooled microwave-irradiated samples in the range of 20-70°C exhibit 62.78 percent inactivation of the enzyme as compared with the controls. In the water-bath heated samples, 96.51 percent of the enzyme was inactivated as compared with the controls. In the other samples (temperature range 16-38°C), there was no significant enzyme inactivation regardless of whether the samples were cooled.

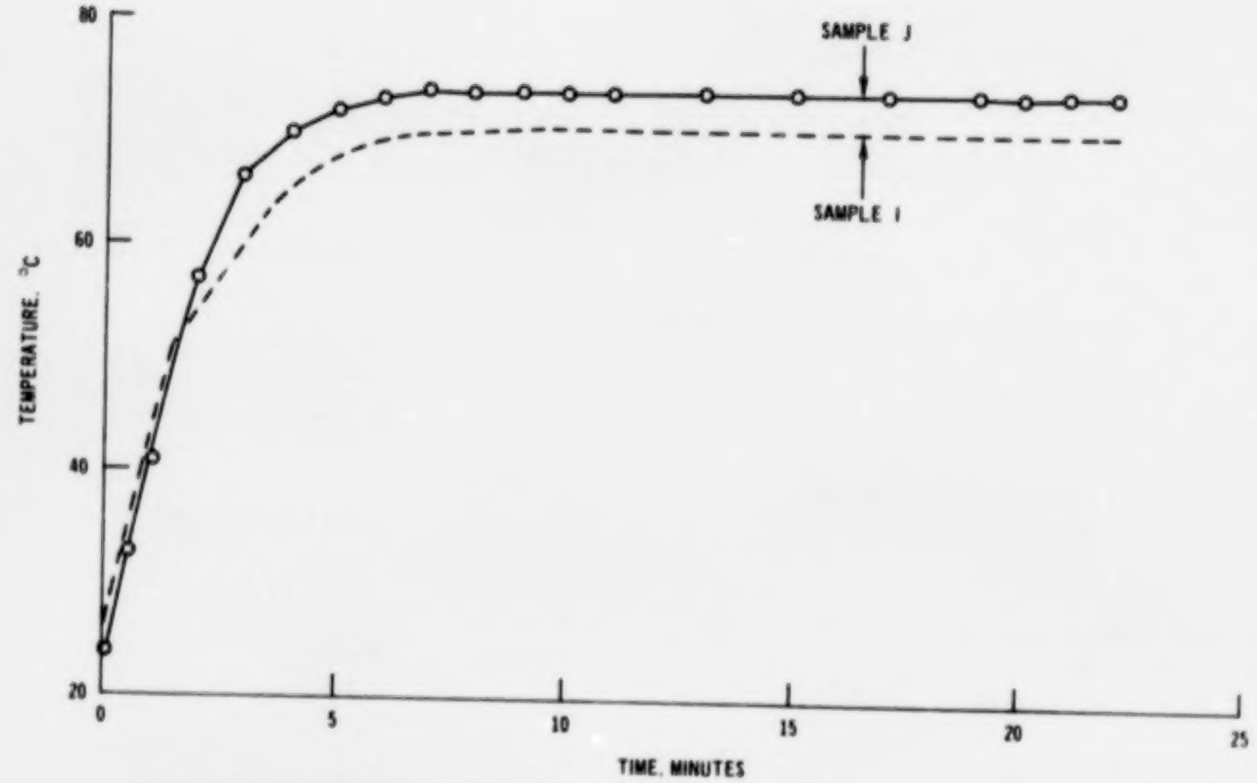

Figure 4. Sample Temperatures During Water-Bath Heating.

Discussion

The results of the in vitro microwave irradiation at 2450 MHz of bacterial alpha-amylase suggest no nonthermal biological effect but clearly indicate a thermal effect of the microwaves. The inactivation of the bacterial alpha-amylase appears to depend on the temperature factor during irradiation. There was no significant inactivation of the enzyme in samples that did not exceed 38° C. The significant inactivation (62.78%) of the thermostable bacterial enzyme as compared with the controls occurred only when the temperature of the samples was raised to about 65° C and maintained at 63.7 ± 11.1° C for 15 minutes during irradiation. The temperature-dependent inactivation of the bacterial enzyme with 2450 MHz microwaves is supported by the significant inactivation of the enzyme (96.51%) by heat at 72.0 ± 1.6° C for 15 minutes.

TABLE 1. INACTIVATION OF BACTERIAL ALPHA-AMYLASE IN VITRO BY 2450 MHz MICROWAVES

Sample	Treatment	Temperature, °C		Units of Enzyme		Enzyme Unaltered, %
		Range	Mean	Sample	Mean[a]	
K	Untreated	22-24 (Ambient)	----	0.5652		
L				0.5824	0.5738x	100.00
A	Irradiated With Cooling	16-20	18.0	0.5927		
B				0.5828		
C				0.5961		
D				0.5849	0.5894x	102.72
E	Irradiated No Cooling	16-38	28.8	0.5923		
F				0.5759	0.5841x	101.80
G	Irradiated No Cooling	20-70	63.7	0.1690		
H				0.2581	0.2135y	37.22
I	Water Bath Heated	20-75	72.0	0.0215		
J				0.0185	0.0200z	3.49

a. Means with different letter postcripts are significantly different at the 5% ($P \leq 0.05$) probability level.

Conclusions

(1) The thermostable bacterial alpha-amylase was significantly inactivated (62.78%) in vitro with 2450 MHz microwaves when the temperature of the enzyme solutions was 63.7 ± 11.1° C during irradiation.

(2) No significant inactivation of the enzyme occurred when the enzyme samples were maintained below 38° C regardless of treatment.

(3) Water-bath heated enzyme samples at the temperature of 72.0 ± 1.6° C brought about significant inactivation (96.51%) of the bacterial alpha-amylase.

(4) The temperature-dependent inactivation of the bacterial alpha-amylase in vitro with 2450 MHz microwaves indicates thermal effect of microwave radiation. There was no suggestion of the nonthermal effect of microwaves in this isolated thermostable enzyme system.

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E. HEMATOLOGICAL RESPONSE IN MICE FOLLOWING EXPOSURE TO 30 OR 250 kV X IRRADIATION

Roger A. Budd, Richard Chiacchierini, James Rolofson, Sarah Jane Smith, and DeWitt G. Hazzard

Introduction

Color television receivers emit x rays in the 10-35 keV energy range (1). Workers have described the physical properties (1-5) and the biological effects (6-10) of x rays produced at voltages at or below 100 kVp. Variation in the effectiveness of various x-ray energies has been attributed to nonuniformity of depth dose (7,8). Although x-ray emission in the lower kV regions may represent a potential biological hazard, we lack documented evidence of radiation injury induced by heavily filtered 30 kV x rays. This study attempts to narrow this gap by comparing the recovery pattern of the mouse hematological system following exposure at 30 kV with the well-documented 250 kV x-ray exposure.

Materials and Methods

Dosimetry

This study used spectra with a maximum energy of 30 keV and mean effective photon energy of 23 keV which closely approximated the overall energy distributions reported by Wang et al. (3). The 30 kV x rays were produced with a high output, thin-window x-ray tube (Machlett OEG-75H); inherent filtration, 0.127 mm beryllium; 0-75 kV constant potential power supply (Kilovolt Corporation, Model KVR-100); aluminum filtration, 3.09 mm; half-value layer, 1.2 mm aluminum. The mean dose rote absorbed at midline was 32.4 rads per minute.

The 250 kV x rays were produced by a constant-potential Westinghouse x-ray machine; 0.44 mm tin, 0.25 mm copper, and 1.0 mm aluminum filtration; half-value layer, 2.52 mm copper. The mean dose rate absorbed at midline was 36.6 rads per minute.

Primary dosimetry was determined by irradiating thermoluminescent dosimeter rods. These had been individually calibrated and were composed of extruded lithium fluoride, TLD-100 (Harshaw Chemical Company). They were implanted both along the central axis and surface of a freshly killed mouse. At 30 kV, an average of 90.5 percent of surface dose was delivered at midline compared to 99.4 percent at midline at 250 kV. An average of 94.5 percent of surface dose was delivered to the spleens of mice at 30 kV compared to 99.8 percent at 250 kV.

The mice were irradiated two at a time in individual plastic tubes rotating at 7.5 revolutions per minute.

Experimental Procedures

Four female C57BL/6J mice, 13 to 16 weeks old, were caged together with food and water available at all times.

To measure erythropoietic activity, the mice were injected intraperitoneally with 2.0 μCi/0.4 ml ^{59}Fe 18 hours before tail blood, spleens, and both femurs were taken.

Measurements were obtained at 1, 2, 4, 8, 16, and 32 days postirradiation. Leukocytes were counted with a Model B Coulter counter. Hematocrits and spleen and body weighing were done by standard methods. Blood, femur, and spleen samples were radioassayed with a Baird Atomic gamma spectrometer (Model 530) in a shielded well NaI (Tl) detector. They were counted for 10 minutes, and the counting error was within ±1.0%.

Statistical Procedures

The experimental design was a 3 x 6 factorial (3 levels of kV and 6 days post-irradiation) in three complete blocks with four replicates per block giving a total of 216 experimental animals. By use of logarithmic transformations on all response variables, except body weights and hematocrits, the variance was equalized. Data for each variable or transformed variable were evaluated by analysis of variance. Dunnetts' procedure (11) was used to compare kV level to control means within post-irradiation days.

Results

The total leukocyte response is shown in Figure 1. Mice irradiated with either 30 or 250 kV had significantly lower total leukocyte counts than controls at 1, 2, 4, 8, 16 and 32 days post-irradiation (all at $P < 0.01$). The differences in effects between 30 and 250 kV, when averaged over all days post-irradiation were not statistically different. After the maximum radiation effect at 2 days post-irradiation, total leukocyte counts for both kV levels increased, but did not reach controls by 32 days post-irradiation.

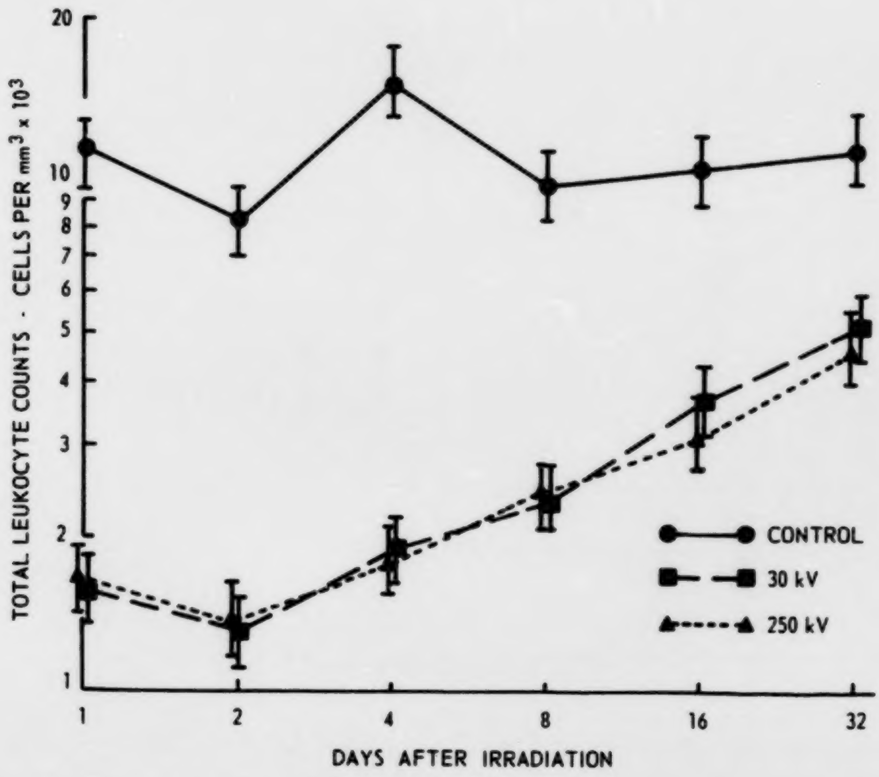

Figure 1. Total Leukocyte Count as a Function of Time Following 240 Rads of X Irradiation (Mean ± 95% confidence limits).

The spleen weights of mice irradiated with 30 or 250 kV were significantly less ($P < 0.01$) than controls on 1, 2, 4 and 8 days post-irradiation (Figure 2). The maximum radiation effect occurred at 2 days post-irradiation. There were no statistically significant differences in spleen weights between animals exposed to 30 and 250 kV, when the effects were averaged over all days post-irradiation. At 16 days post-irradiation spleen weights of irradiated mice were similar to the control weights.

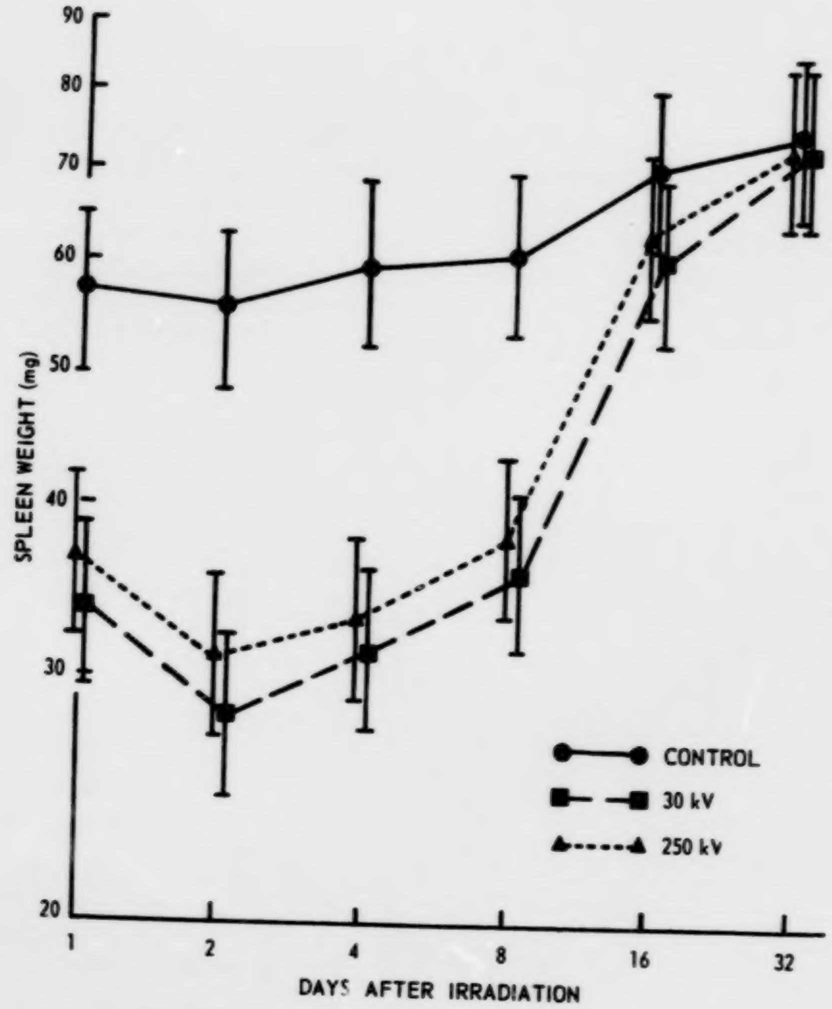

Figure 2. Spleen Weights as a Function of Time Following 240 Rads X Irradiation (Mean ± 95% confidence limits).

Both 30 and 250 kV significantly decreased ($P < 0.01$) ^{59}Fe incorporation into erythrocytes at 1, 2, and 4 days post-irradiation (Figure 3). The effects of the two kV levels were not statistically different when averaged over all days post-irradiation. The maximum radiation effect was at 2 days post-irradiation, and irradiated mice attained similar values to the controls by 8 days post-irradiation.

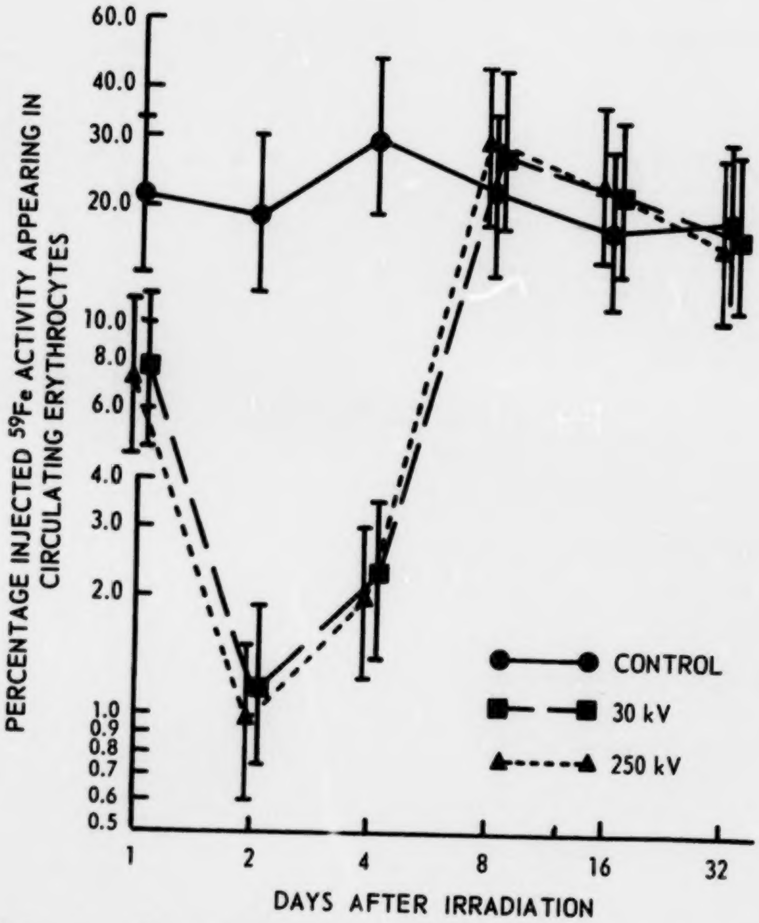

Figure 3. Incorporation of ^{59}Fe into Circulating Erythrocytes as a Function of Time Following 240 Rads X Irradiation (Mean ± 95% confidence limits).

Figure 4 shows the ^{59}Fe uptake into femoral bone marrow. There were no statistically significant differences between the effects of 30 and 250 kV, when averaged over all days post-irradiation. At one day post-irradiation, the 250 kV level was significantly higher ($P < 0.05$) than the controls. By two days post-irradiation both the 30 and 250 kV values were significantly lower than the controls. The maximum radiation effect occurred at 2 days post-irradiation, and by 4 days post-irradiation femoral ^{59}Fe uptakes of irradiated mice were similar to those of the controls.

Hematocrits of mice irradiated with 250 kV were significantly less ($P < 0.05$) than controls at 2 days post-irradiation. At 4 and 8 days post-irradiation both the 30 and 250 kV were significantly lower ($P < 0.01$) than the controls (Figure 5). The maximum radiation effect occurred at 4 days post-irradiation and irradiated mice attained similar values to the controls by 16 days post-irradiation. There were no statistically significant differences between the effects of 30 and 250 kV, when averaged over all days post-irradiation.

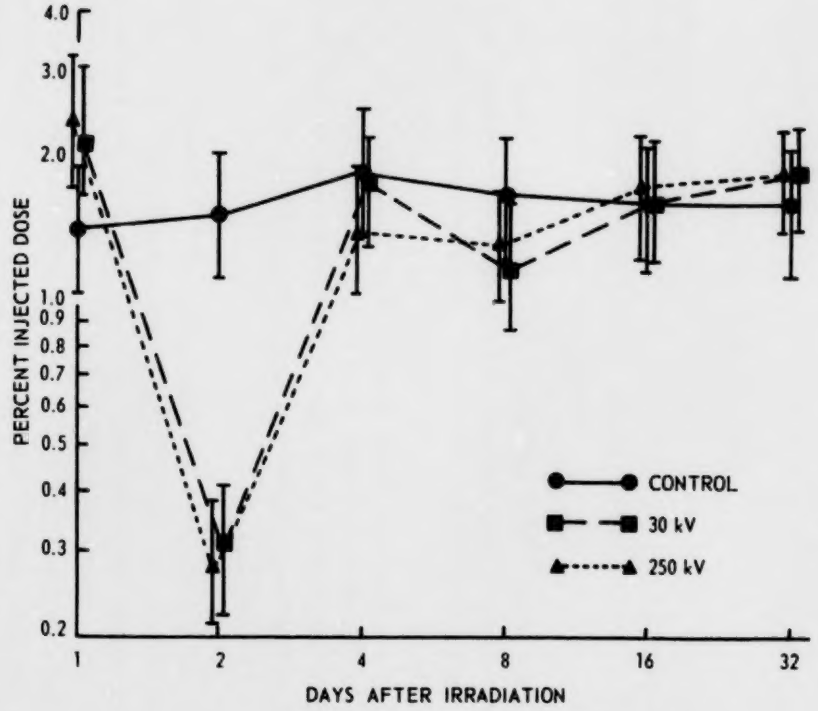

Figure 4. Femoral Bone Marrow Uptake of ^{59}Fe as a Function of Time Following 240 Rads of X Irradiation (Mean ± 95% confidence limits).

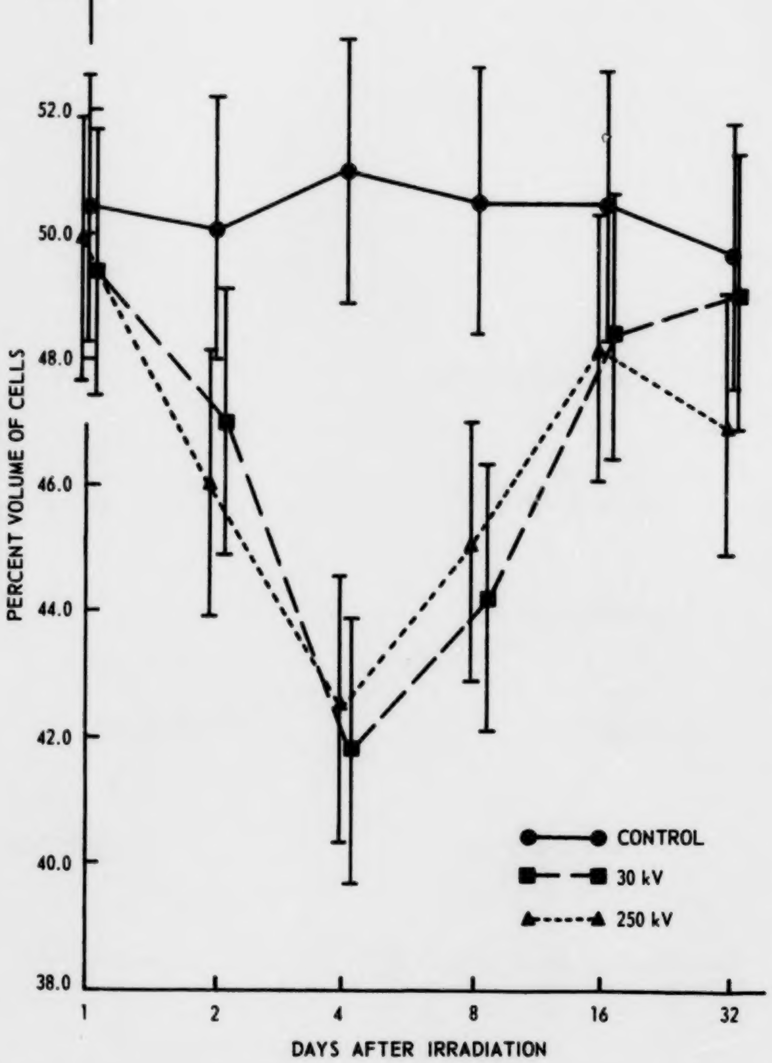

Figure 5. Hematocrit Values as a Function of Time Following 240 Rads of X Irradiation (Mean ± 95% confidence limits).

There were no statistically significant radiation effects of either body weights or splenic uptake of ^{59}Fe.

Conclusions

The maximum radiation effect occurred between 1 and 4 days postirradiation. All variables affected by radiation, except leukocyte counts, recovered between 4 and 32 days postirradiation. The leukocyte counts had not completely recovered by 32 days postirradiation, but the counts were still increasing at that time.

These results indicate that there is no apparent difference in the injury-recovery pattern following 240 rads of 30 or 250 kV x irradiation on the measurements of the mouse hematological system selected for this study.

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F. BLOOD CELL RECOVERY OF MOUSE BONE MARROW AND SPLEEN FOLLOWING 240 RADS OF 30 OR 250 kV X IRRADIATION

Roger A. Budd and James N. Shively

The purpose of this study was to compare the recovery patterns of splenic and bone marrow hematopoietic tissue following injury by 30 or 250 kV x irradiation.

Female C57BL/6J mice received 240 rads of either 30 or 250 kV x irradiation. The spleen and both femurs were obtained at 1, 2, 4, 8, 16, and 32 days postirradiation. Tissues were fixed in 10% buffered formalin and slides were prepared using standard histological procedures. The experimental design and irradiation conditions are described on p. 154.

The first day after irradiation, spleen germinal centers were sparse and each contained several necrotic cells. The cortex was absent. The bone marrow was 3+ hypocellular and mature myeloid forms predominated. There appeared to be more immature forms in bone marrows exposed to 30 kV than with 250 kV. Sinuses contained 3+ blood; overall, in mice exposed to 30 kV, the blood cells were as altered or perhaps a little less altered than in mice exposed to 250 kV.

Two days after irradiation spleens were atrophic. Although germinal centers were sparse, they were populated. A few of the cells were necrotic. Bone marrows had 3 to 4+ hypocellularity and myeloid elements seemed to predominate over erythroid elements. Sinuses contained 3 to 4+ blood. Overall, damage with 30 and 250 kV was similar.

Four days after irradiation, spleens were still atrophic. The germinal centers were sparse, but were populated with lymphoid cells. A few necrotic cells were in these centers. The bone marrows 2 to 3+ hypocellular with a predominance of erythroid precursors. There was considerable pooling of blood in the sinusoids. Gross cellular damage induced by 30 kV was similar or perhaps a little less severe than 250 kV.

By eight days after irradiation, spleens were recovering. Germinal centers were populated, but the cortex was still sparse. The bone marrow was 1+ hypocellular, but was recovering. Erythrocytic precursors were prominent. There were no apparent differences between tissue irradiated with either 30 or 250 kV.

Sixteen days after irradiation, spleens were recovered. There was a slight excess of erythrocytic precursors in the bone marrow. Generally we could not differentiate between tissue irradiated with 30 or 250 kV.

By thirty-two days after irradiation, spleens and bone marrows had recovered and there was no apparent difference between tissue irradiated with 30 or 250 kV.

Conclusion

Generally, the degree of femoral bone marrow and splenic cellular damage and their subsequent recovery patterns following irradiation with 30 and 250 kV was comparable for both groups.

G. HEMATOLOGICAL RESPONSE OF FETAL RATS FOLLOWING 2450 MHz MICROWAVE IRRADIATION

Roger A. Budd, John Laskey, and Catherine Kelly

Objective

The object of this preliminary study was to determine the radiosensitivity of the fetal hematological system following *in utero* microwave irradiation.

Materials and Methods

Pregnant Sprague-Dawley rats were exposed one at a time to whole-body microwave irradiation at 15 days gestation. Exposures were performed in an anechoic chamber using 100 mW/cm^2 of 2450 MHz continuous-wave microwave irradiation. Animals were exposed for 8.5 minutes in a cylindrical lucite container placed horizontally to the incident beam. The distance from the edge of the transmitting horn to the animal midline was 100 cm. Exposures were made in still air at ambient temperature of 74.5° F and 66 percent relative humidity.

Under these conditions this sublethal dose increased the rectal temperatures of the pregnant rats 4.2° C above that of the controls. Hematological changes were measured in the pregnant rats at 4 hours, 24 hours, and 5 days postirradiation (shortly before the fetuses were removed). Body and spleen weights, and hematological changes were measured in the fetal rats at 20 days gestation.

Results

No significant differences were found between the control and microwave exposed pregnant rats for body weights, total leukocyte counts, erythrocyte counts, hematocrits, or hemoglobin values.

Body weights and hematological variables for control and microwave irradiated fetal rats are summarized in Table 1.

Microwave irradiated fetuses had significantly lower spleen weights ($P < 0.05$), total leukocyte counts ($P < 0.01$), and somewhat lower hemoglobin values ($P < 0.10$) than controls. No appreciable differences were observed between microwave irradiated fetuses and their controls for body weights, ^{59}Fe uptakes in blood or fetal liver, nucleated erythrocyte counts, erythrocyte counts, hematocrits, or fetal resorption.

Conclusion

This preliminary study indicates fetal sensitivity to high doses of microwave irradiation at 15 days gestation as demonstrated by leukopenia, decreased spleen weights, and hemoglobin values. These limited data suggest that fetuses may be more sensitive to microwave irradiation than their mothers. In order to understand the nature of this injury, additional experiments should be performed under controlled environmental conditions using greater numbers of experimental animals and lower microwave doses.

TABLE 1. BODY WEIGHTS AND HEMATOLOGICAL VARIABLES AT 20 DAYS GESTATION IN FETAL RATS MICROWAVE IRRADIATED AT 15 DAYS GESTATION

Measurement	Control[a]	Microwave Irradiated[a]	Student's t-test[b]
Body Weight, g	3.32 ± 0.90	3.30 ± 0.05	N.S.
Spleen Weight, mg	7.4 ± 0.5	4.7 ± 0.8	$P < 0.05$
Total Leukocyte Count, 10^3 cells/mm^3	3.50 ± 0.21	2.50 ± 0.18	$P < 0.01$
^{59}Fe Uptake (Blood), cpm	4,903 ± 468	5,329 ± 506	N.S.
^{59}Fe Uptake (Liver), cpm	24,973 ± 2,453	24,096 ± 2,006	N.S.
Nucleated Erythrocytes in Blood, cells/mm^3	24.70 ± 3.43	23.10 ± 3.15	N.S.
Total Erythrocytes, 10^3 cells/mm^3	870 ± 63	890 ± 59	N.S.
Hematocrit, percent volume of cells	45.7 ± 1.5	44.6 ± 2.1	N.S.
Hemoglobin, gram percent	12.11 ± 0.47	11.48 ± 0.32	$P < 0.10$
Mean Corpuscular Volume, μ^3	550.2 ± 55.0	518.2 ± 43.7	N.S.
Mean Corpuscular Hemoglobin, $\mu\mu$g	145.2 ± 13.6	133.5 ± 9.6	N.S.
Mean Corpuscular Hemoglobin Conc., %	26.6 ± 0.9	26.1 ± 1.3	N.S.

a. All values ± standard error of the mean. Eight fetuses, obtained from two dams, were used in each group.

b. N.S. denotes that differences between the control and microwave irradiated groups were not statistically significant.

H. KINETICS OF MOUSE HEMATOPOIETIC COLONY-FORMING UNITS FOLLOWING INJURY BY 2450 MHz MICROWAVE IRRADIATION

Roger A. Budd, John Laskey, and Mary Howes

Introduction

The enumeration of transplanted hematopoietic colony-forming units (CFU) in the spleens of recipient mice is assumed to provide an assay of stem cells (1,2). The purpose of this preliminary study was to determine population changes of spleen and bone marrow CFU's following a high dose of microwave irradiation.

Materials and Methods

Donor mice (BALB/c, 24-26 weeks old) were either microwave or sham irradiated. Cell suspensions were prepared from both splenic and bone marrow tissue. Aliquots of either bone marrow or splenic donor cells were injected into recipient mice of the same strain and age at 1 or 2 days following microwave exposure. The recipient mice were previously x-irradiated with 700 rads to suppress endogenous CFU's. The recipient mice were killed 10 days after the x irradiation. The spleens were removed, fixed in Bouins' solution and visually inspected to enumerate CFU's and radioassayed for ^{59}Fe to estimate erthropoietic activity.

Irradiation

The donor mice were microwave irradiated two at a time in individual plastic tubes rotating at 7.5 revolutions per minute. The mice were exposed for 5 minutes to 100 mW/cm^2 of 2450 MHz continuous wave microwave irradiation. The distance from the edge of the transmitting horn to animal midline was 100 cm. Exposures were made in still air at an ambient temperature of 72.5°F and 51 percent relative humidity. Under these conditions this microwave dose raised rectal temperatures 6.4°C above controls and killed 75 percent of the mice within two days post-irradiation.

Results

Table 1 summarizes the concentration of colony-forming units (CFU) in the spleens and femoral bone marrow following microwave irradiation. Bone marrow CFU's were significantly decreased by microwaves at one and two days post-irradiation (both at $P < 0.01$). At one day post-irradiation the microwave group was approximately 45 percent of the control and at two days post-irradiation the microwave group was approximately 66 percent of the control.

TABLE 1. CONCENTRATION OF COLONY-FORMING UNITS (CFU) OBTAINED FROM THE SPLEENS OR FEMORAL BONE MARROWS OF MICE IRRADIATED WITH 2450 MHz MICROWAVES

Group	CFU per 10^5 Nucleated Cells[a]	
	Femoral Bone Marrow	Spleen
	One Day Post-Irradiation	
Control	24.44 ± 1.71 (10)	0.58 ± 0.09 (10)
Microwave	11.12 ± 0.65 (10)	0.67 ± 0.12 (10)
Student's t-test	$P < 0.01$	Not significant
	Two Days Post-Irradiation	
Control	(24.44 ± 1.71)[b] (10)	0.55 ± 0.10 (7)
Microwave	16.16 ± 1.01 (10)	0.48 ± 0.14 (10)
Student's t-test	$P < 0.01$	Not significant

a. Values ± standard error of the mean. Number in parentheses denotes number of recipient mice in each group.

b. Estimated control value based on value obtained at 1 day post-irradiation.

TABLE 2. RELATIVE ERYTHROPOIETIC ACTIVITY OF RECIPIENT SPLEENS THAT RECEIVED EITHER SPLEEN OR FEMORAL BONE MARROW CELLS FROM MICE IRRADIATED WITH 2450 MHz MICROWAVES

Group	Percentage ^{59}Fe Uptake in Spleens per 10^5 Nucleated Cells Injected[a]	
	Femoral Bone Marrow	Spleen
	One Day Post-Irradiation	
Control	8.99 ± 0.82 (10)	0.26 ± 0.03 (10)
Microwave	3.25 ± 0.52 (9)	0.32 ± 0.04 (10)
Student's t-test	$P < 0.01$	Not significant
	Two Days Post-Irradiation	
Control	(8.99 ± 0.82)[b] (10)	0.21 ± 0.04 (7)
Microwave	5.37 ± 0.77 (9)	0.23 ± 0.03 (9)
Student's t-test	$P < 0.01$	Not significant

a. Values ± standard error of the mean. Number in parentheses denotes number of recipient mice in each group.

b. Estimated control value based on value obtained at one day post-irradiation

No statistically significant differences were found in splenic CFU's at 1 or 2 days post-irradiation.

Table 2 summarizes the relative erythropoietic activity of recipient spleens that received either spleen or bone marrow cells from microwave irradiated mice. In mice that received bone marrow cells, the splenic uptake of ^{59}Fe was significantly decreased by microwaves at 1 and 2 days post-irradiation (both at $P < 0.01$). The microwave group was approximately 36 percent of the control at 1 day post-irradiation and at 2 days post-irradiation it was 60 percent of the control.

In mice that received spleen cells, no statistically significant differences were found between the microwave and control groups, at either 1 or 2 days post-irradiation.

Conclusions

Stem cell function was decreased by acute microwave irradiation as reflected by decreased CFU's. Presumably damage to these primitive colony-forming cells would eventually impair normal hematopoiesis because normal differentiation and renewal of cell populations could not occur. Mice that received microwave irradiated bone marrow cells showed impaired erythropoiesis, as measured by splenic uptake of ^{59}Fe. These results show a correlation between decreased hematopoiesis in general and decreased erythropoiesis in particular.

The fact that microwave irradiated spleen cells did not show a decrease in either erythropoiesis or stem cell function indicates a differential microwave sensitivity between splenic and bone marrow tissue. Further experiments are planned to determine if the donor bone marrow cells themselves are more sensitive than spleen cells or whether the location of the tissue and its blood supply is responsible. Additional experiments are also planned to determine stem cell sensitivity using lower sublethal microwave doses.

References

1. Till, J.E., and E.A. McCulloch. 1961. A direct measurement of the radiation sensitivity of normal mouse cells. Radiation Res. 14: 213-222.

2. Till, J.E., and E.A. McCulloch. 1964. Repair processes in irradiated mouse hematopoietic tissue. Ann. N.Y. Acad. Sci. 114: 115-125.

I. LETHAL DOSE OF 2450 MHz MICROWAVE IRRADIATION AT VARIOUS POWER DENSITIES IN THE SPRAGUE-DAWLEY RAT (A Preliminary Report)

John Laskey

Male Sprague-Dawley rats (150 to 250 grams), held in extensively perforated lucite containers, were exposed to microwaves in an anechoic chamber approximately 5 by 6 by 7 feet deep built of Emerson and Cumings CVB-24 (-36 dB ± 2 dB reflectivity) anechoic material. There was no anechoic material on the floor. Microwaves generated with a PPS-2.5 AS Varian generator capable of producing 3.0 kW at 2450 MHz were broadcast through a 10 dB scientific Atlanta standard gain pyramidal horn. Animals were placed horizontally 100 cm from the aperture of the transmitting horn on the main beam axis. In the absence of the target, the average power densities used as measured with a tuned dipole were 58, 115, 175, 232, or 293 mW/cm^2 ± 15 percent. Additional measurements showed that, at up to 10 cm off the axis, the power density falls by 5 percent. The detecting element, a PRD type N 685-2 thermoelectric power head, was connected to a PRD type 6685 thermoelectric power meter. Between the dipole antenna and the detector head was a RG-8/u cable measured at 6.5 dB attenuation at 2450 MHz. Rectal temperatures were taken prior to exposure. Animals were observed during exposure, and the time of death was recorded to the nearest 0.5 minute when respiration ceased. Rectal temperatures were taken within 1 minute following cessation of respiration. The data are reported in Table 1.

J. PROGRESS REPORT ON 2450 MHz IRRADIATION OF PREGNANT RATS AND THE EFFECT ON THE FETUS

John Laskey, Daniel Dawes, and Mary Howes

This is a report of a pilot study designed to test the effect of 2450 MHz microwave irradiation delivered to pregnant rats on the unborn pups. Results of this study will be used to design future *in utero* studies.

Materials and Methods

Time-pregnant Sprague-Dawley rats weighing 175 to 275 grams were shipped to our laboratory on the day they were determined to be sperm positive (day of conception is day 0). Except for the day of exposure, animals were maintained in our animal facilities at 21 ± 1°C and 40 to 70 percent relative humidity. Pregnant animals were held in cylindrical lucite containers which had been preforated extensively with 1/4 inch holes to allow air circulation during microwave exposure. The

TABLE 1. TIME-TO-DEATH IN RATS EXPOSED TO MICROWAVES AT VARIOUS POWER DENSITIES

Exposure Power Density, mW/cm^2	Animals Exposed	Mean Weight, g	Rectal Temperature, °C		Lethal Dose, min.	Ambient Temperature, °C	Relative Humidity, %
			Pre-Exposure	Post-Exposure			
58	8	198±2	37.4±0.1	45.0±0.1	24.4±1.2	26.5±0.4	55.1±1.8
115	43	212±3	37.5±0.1	45.3±0.2	10.8±0.2	25.9±0.3	64.6±1.1
175	14	190±4	37.6±0.1	46.4±0.2	7.4±0.3	25.6±0.5	57.2±0.8
232	4	202±4	37.5±0.2	48.2±0.4	6.0±0.2	28 -	48 -
293	4	167±6	37.6±0.1	46.0±0.8	3.8±0.3	26 -	56 -

animals were not restrained but the containers allowed the animals only minimal movement.

The animals were exposed in an anechoic chamber approximately 10 by 20 by 8 feet, built of Emerson and Cumings CVB-24 (-36 dB ± 2 dB reflectivity) anechoic material. Microwaves, generated with a PPS - 2.5 AS Varian generator capable of producing 3.0 kW at 2450 MHz, were broadcasted through a 10 dB Scientific Atlanta standard gain pyramidal horn. Animals were placed horizontally 100 cm from the aperture of the transmitting horn on the main beam axis. In the absence of the target, the average power density was measured with a tuned dipole to be 100 mW/cm^2 ± 15 percent. Additional measurements showed that, at up to 10 cm away from the axis, the power falls by about 5 percent. The detecting element, a PRD type N685-2 thermoelectric power head, was connected to a PRD type 6685 thermoelectric power meter. Between the dipole antenna and the detector head was an RG-8/u cable measured at 6.5 dB attenuation at 2450 MHz.

Pregnant animals were exposed during the preimplantation stage, day 2 or day 2 and day 5; the postimplantation stage, day 8; or in the postorganogenesis stage, day 13 of gestation. Each animal was exposed for 8 to 13 minutes. Rectal temperatures were taken on each animal before exposure and within 1 minute after exposure. Ambient temperature and relative humidity on the day of exposure were noted. Following exposure the animals were returned to the animal facility until sacrifice on day 19 of gestation.

Sham irradiated animals were placed in the lucite containers and their rectal temperatures were determined initially and following 13 minutes in the container. Animals in the control group were maintained in the animal facility until sacrifice.

On day 19 of gestation the animals were sacrificed by decapitation and the fetuses removed, counted, weighed and examined for gross pathology; the numbers of resorptions and nonpregnant animals were noted. Some animals were selected for histological examination and for cell-free incorporation of ^{14}C phenylalanine.

Results

Figure 1 presents a typical temperature profile that is valid for a particular ambient temperature and relative humdity.

Exposure conditions with the postimplantation and postorganogenesis groups show significant ($P <.05$) increases in relative humidity during exposure (Table 1).

When compared to the preimplantation group, this increase in relative humidity is reflected in a significantly higher ($P < 0.05$) mean rectal temperature postexposure for these groups (Table 2).

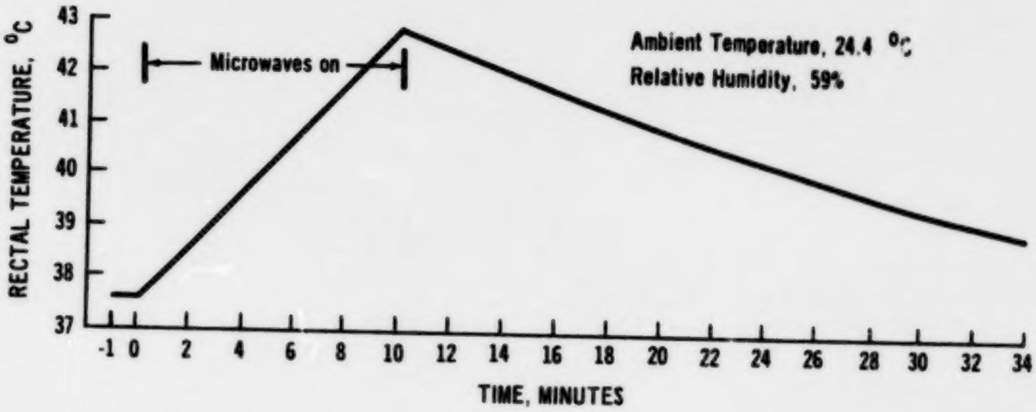

Figure 1. Typical Temperature Profile of Pregnant Rats Exposed to 2450 MHz Microwaves. Power density 100 mW/cm^2 ± 15% 100 cm from the aperture of the transmitting horn on the main axis during 10 minute exposure and 24-minute cooling period.

TABLE 1. AMBIENT CONDITIONS DURING MICROWAVE EXPOSURE

	Ambient Temperature, °C		Relative Humidity, %	
	Mean	Range	Mean	Range
Control	-	-	-	-
Sham	22.3	19.5-22.8	38.1	23-65
Preimplantation	22.9	21.6-25.4	19.5	15-40
Postimplantation	23.3	22.8-25.6	68.0	55-72
Postorganogenesis	23.2	22.8-23.9	69.0	- -69

TABLE 2. RECTAL TEMPERATURES BEFORE AND AFTER EXPOSURE TO 2450 MHz MICROWAVE IRRADIATION

	No. of Dams Examined	Mean Rectal Temperature, C			
		Pre-exposure		Post-exposure	
Control	-	-	-	-	-
Sham	60	37.5	0.1	38.0	0.1
Preimplantation	24	37.7	0.1	41.4	0.1
Postimplantation	26	37.6	0.2	42.8	0.1
Postorganogenesis	21	37.5	0.2	42.3	0.1

In addition, previous work with microwave irradiation in this laboratory has indicated that increases in relative humidity may cause as much as a 30 percent decrease in the lethal dose (in minutes) with other exposure conditions constant.

The frequency of nonpregnant dams (Table 3) did not show a significant increase in any of the groups examined when compared with the control or sham irradiated groups.

Fetal resorption was significantly greater ($P < 0.05$) in the preimplantation group when measured either as the number of resorptions observed or as the number of dams with resorptions (Table 4).

TABLE 3. NONPREGNANT DAMS FOLLOWING EXPOSURE TO 2450 MHz MICROWAVE IRRADIATION

	Dams Examined	Dams Not Pregnant
Control	42	4
Sham	60	7
Preimplantation	24	5
Postimplantation	26	3
Postorganogenesis	21	4

TABLE 4. RESORPTION FOLLOWING EXPOSURE TO 2450 MHz MICROWAVE IRRADIATION

	Dams Examined	Resorptions Observed	Dams With Resorptions
Control	38	5	3
Sham	53	13	10
Preimplantation	19	4	1
Postimplantation	23	32	10
Postorganogenesis	17	7	5

There did not appear to be any significant decrease in litter size in any of the groups examined (Table 5). Mean fetal weights of the postimplantation and postorganogenesis groups show a significant ($P < 0.05$) decrease when compared to either the sham irradiated or control groups.

All fetuses were examined upon removal from the uterus and no gross malformations were noted. A few fetuses from each group were examined histologically and did not show any significant pathology.

Litters selected for cell-free protein synthesis showed a reduction in brain weight comparable with the reduction in body weight following exposure in the postimplantation and postorganogenesis stages of gestation (Table 6).

TABLE 5. MEAN NUMBER OF FETUSES PER LITTER AND MEAN FETAL WEIGHT ON DAY 19 OF GESTATION

	Dams Examined	Mean Fetuses per Litter	Mean Fetal Weight, g
Control	38	10.32 ± 0.40	2.08 ± 0.03
Sham	53	10.49 ± 0.47	2.03 ± 0.03
Preimplantation	19	9.95 ± 0.41	2.05 ± 0.03
Postimplantation	23	9.73 ± 0.41	1.81 ± 0.05
Postorganogenesis	17	9.18 ± 0.78	1.92 ± 0.04

TABLE 6. INCORPORATION OF ^{14}C-PHENYLALANINE INTO PROTEIN IN A CELL-FREE SYSTEM DERIVED FROM BRAIN TISSUE ON DAY 19 OF GESTATION

	Litters Pooled	Fetuses	Mean Fetal Wt., g	Mean Brain Wt., g	^{14}C-Phe Incorporation dpm/g Brain
Control	2	22	2.04	0.126	62,000
Postimplantation	2	18	1.84	0.117	51,000
Postorganogenesis	2	20	1.89	0.118	51,000

Summary

Exposure of pregnant rats to from 75 to 95% of the lethal dose of 2450 MHz microwaves (100 mW/cm^2 at 100 cm) revealed:

1) No significant differences in the number of nonpregnant animals in the groups examined.

2) A significant increase in the number of resorptions in the dams exposed on day 8 of gestation.

3) A significant decrease in the weight of fetuses from dams exposed on day 8 and day 13 of gestation.

4) No significant pathology in any of the animals examined.

5) A 20% reduction of brain cell-free incorporation of ^{14}C phenylalanine into protein from fetuses exposed on day 8 and day 13 of gestation.

K. THE EFFECT OF X-IRRADIATION ON MITOCHONDRIAL DNA, RNA, AND PROTEIN SYNTHESIS

Melvin E. Stratmeyer, Lawrence R. Simmons, and Katherine M. Selby

In recent years it has been demonstrated that mitochondria contain DNA that has physical, chemical and enzymatic characteristics different from those of nuclear DNA. Although the function of this mitochondrial DNA is unknown at the present time, it is likely that it is an integral part of the control mechanism for the synthesis of many of the enzymes found in mitochondria. It is presently accepted that the enzymes localized in the mitochondria are responsible for catalyzing the reactions of the Krebs citric acid cycle and oxidative phosphorylation by which pyruvate is oxidized to carbon dioxide and water with the concomitant release of energy (in the form of ATP). Thus the mitochondria supply the energy by which a cell functions. This project proposes to determine what, if any, are the effects of irradiation upon mitochondrial DNA, RNA, and protein metabolism by measuring the uptake of selected radioactive precursors into these various fractions. Any alterations in the metabolism of these fractions should be an indication of radiation-induced imbalance of the homeostatic mechanisms of the cell, which might eventually result in a macroscopic lesion.

In Vitro Incorporation of ^{3}H-Thymidine

Twenty-four hours after irradiation with 180 rads of 250 kVp x rays (or sham irradiation of the control animals), 180-200 g Sprague-Dawley rats were sacrificed and their livers excised and homogenized in cold 0.25 M sucrose-0.001 M EDTA using a "Virtis 45" homogenizer. The homogenates were then strained through gauze and centrifuged at 1,080 times gravity (xg) for 10 minutes at 4°C. The supernatant was decanted and the centrifugation was repeated twice more. The mitochondria were then isolated by centrifugation at 10,800 xg for 10 minutes at 4°C. The mitochondria were washed five times by suspension in the 0.25 M sucrose (without EDTA) and centrifuged at 8,000 xg for 10 minutes at 4°C. The mitochondria were then incubated in a system slightly modified from that used by Mitra and Bernstein (1) at 37° for 1 hour.

After incubation the DNA, RNA, and protein were precipitated with cold perchloric acid. After washing the pellet three times with cold perchloric acid, the DNA was hydrolyzed using 0.5 N perchloric acid and incubated at 96° for 45 minutes. The supernatant containing the hydrolyzed DNA was decanted and the protein pellet washed three times with 0.5 N perchloric acid. The protein pellet was then put into alkaline tartrate and measured using a modified Lowry method on a Technicon Autoanalyzer. An aliquot of the hydrolyzed DNA was taken to determine the DNA concentration using a slightly modified method by Burton (2). An aliquot of the hydrolyzed DNA was also counted in a Packard Tri-Carb scintillation counter.

As the data obtained from earlier studies seemed to be rather erratic, it was recently decided to perform partial hepatectomies on the rats prior to sacrifice in order to bring a large portion of the regenerating liver cells into synchrony. The partial hepatectomies were performed 3 hours postirradiation and the animals were sacrificed 24 hours postirradiation. The data obtained thus far are shown in Table 1. As of this time, no statistics have been performed on the data, but it would appear that the in vitro ^{3}H-thymidine incorporated into the mitochondrial DNA from regenerating rat liver is greater in the x-irradiated animals than in the sham-irradiated animals.

In Vivo Incorporation of ^{14}C-Glycine

In this study, the animals were injected with ^{14}C-glycine 22 hours after irradiation and sacrificed 24 hours postexposure. The mitochondria were isolated in the manner previously described. The lipid, RNA, DNA, and protein fractions were then separated. At this time all attempts to separate DNA, completely uncontaminated by protein, have been unsuccessful. Methods in the literature which supposedly produce pure fractions of RNA and DNA have been found by this group to yield fractions contaminated with protein; the DNA fraction is especially heavily contaminated. Until this problem is resolved no valid data can be collected.

TABLE 1. EFFECT OF 180 RADS OF 250 KVP X RAYS ON THE IN VITRO INCORPORATION OF ^{3}H-THYMIDINE INTO MITOCHONDRIAL DNA OF REGENERATING RAT LIVER

	dpm/mg Protein		Increase of Irradiated to Control, %
	Irradiated	Control	
Run 1	302	118	256
Run 2	142	65	219

References

1. Mitra, R.S., and I.A. Bernstein. 1970. Thymidine incorporation into deoxyribonucleic acid by isolated rat liver mitochondria. J. Biol. Chem. 245:1255-1260.
2. Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-323.

L. EFFECT OF ULTRASOUND ON THE GROWTH AND METABOLISM OF A CHINESE HAMSTER TISSUE CULTURE CELL LINE (A Preliminary Report)

Richard K. Fred and Bonnie Bowen

There have been reports in the literature indicating that ultrasound can alter the growth and metabolism of certain plants. In our laboratory we are studying the effect of ultrasonic energy on the V79-753-B3 fibroblast, the same cells used and well characterized by Mortimer Elkind of the National Cancer Institute in all of his studies of the biological effects of ionizing radiation. We have been specifically interested in the ultrasonic alteration of the de novo synthesis of protein and the impariment of cell division as measured by the ability of individual cells to form colonies.

The preliminary experimental investigations have utilized ultrasonic energy at 0.669 MHz and approximately 1 to 4 W/cm^2. This frequency is being used because it is the primary resonant frequency of our irradiation transducer. The equipment which we are using is shown in Figure 1. The amplifier is connected to an oil-cooled transducer. The output parameters of the amplifier, frequency, voltage, and current into the transducer, are monitored. The temperature of the brass acoustic compressor is also monitored. The cells are irradiated in sterile lucite petri dishes which are acoustically coupled to the acoustic compressor by an ultrasonic coupling gel.

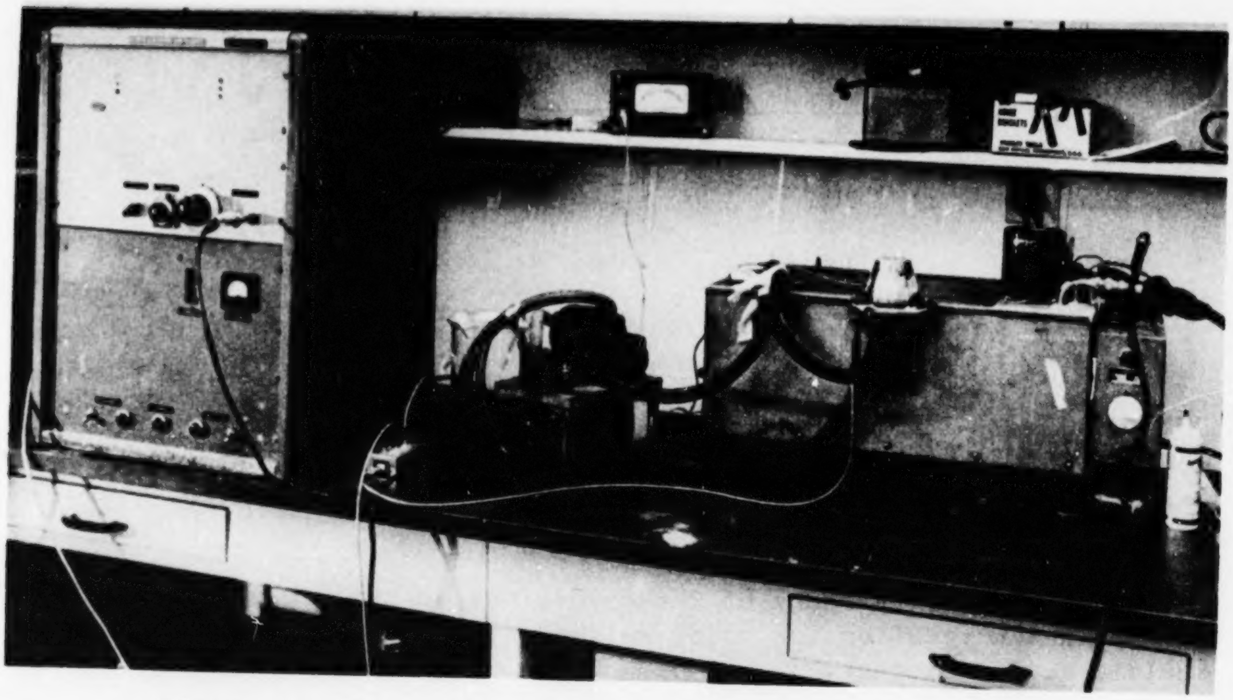

Figure 1. Ultrasonic Generator (left) with Auxiliary Pump (center) and Heat Exchanger (right).

We have often found a decrease in the rate of incorporation of radioactive labeled amino acids into proteins with cell suspensions exposed to ultrasound. In some experiments we observed no effect of ultrasound on amino acid incorporation. Experimental parameters are being examined to pinpoint the source of this inconsistency.

We have evidence that exposure to ultrasound decreases the viability of the tissue culture cells as measured by their ability to form colonies. The dose-effect relationship, which we have obtained in most experiments, indicated a definite biological effect which was independent of exposure dose. These data are presented in Table 1. This puzzling result may have been solved by results from a very recent experiment where the cell multiplicity was kept very near unity (Table 2). In earlier experiments we had not determined the cell multiplicity and feel that it could have been as high as 1.4, in which case our initial dose-effect points could have been as much as 40% in error.

TABLE 1. PERCENT SURVIVAL (COMPARED TO CONTROLS)
IN INITIAL EXPERIMENTS
WITHOUT REGARD TO CELL MULTIPLICITY

Exp. No.	Minutes of Exposure to Ultrasound				
	5	10	20	30	40
19	90	75	68	40	-
24	78	122	87	113	113
27	93	53	67	81	69
32	105	93	99	81	95
35	72	85	-	68	101
37	105	134	95	100	90
38	83	74	80	100	56

TABLE 2. PERCENT SURVIVAL (COMPARED TO CONTROLS)
IN EXPERIMENTS
WITH CELL MULTIPLICITY KEPT BELOW 1.10

Exp. No.	Minutes of Exposure to Ultrasound					
	2	5	10	15	20	30
41	-	37	5	-	0	0
42	62	49	26	9	4	3

M. EFFECTS OF X IRRADIATION ON THE DEVELOPING NERVOUS SYSTEM

T. G. Zaremba, R. P. Felten, A. Lamanna, and M. L. Shore

Introduction

Many studies have been published demonstrating morphological, histological, and functional abnormalities in the nervous system of offspring following in utero x irradiation (1,2). Such findings provide relatively gross indicators of radiation damage, but they provide little information on the underlying causes of damage, no basis for the prediction of long-term effects, and no means for evaluating possible repair mechanisms. To obtain such information, it is necessary to investigate radiation effects on the nervous system at the molecular level. Alteration of DNA structure provides the clearest example of radiation damage which carries the potential for long-term effects. Control of cellular activity by DNA is mediated by the controlled synthesis of proteins, thus the investigation of protein synthesis becomes a critical endpoint for assessing radiation damage and repair during development. Previous studies in this laboratory have indicated that *in utero* x irradiation interferes with the development of liver enzymes at birth (3) and causes impaired incorporation of amino acid into protein in the fetus (4). Little information is available on the effects of x irradiation on molecular processes in the developing nervous system. Reports have appeared showing a decrease in central nervous system enzyme activity (5-7) and changes in amino acid composition of brain proteins (8) following *in utero* x irradiation.

The present study was designed to further elucidate the effect of *in utero* irradiation on parameters associated with protein synthesis in the developing brain.

Method

Pregnant Sprague-Dawley rats were obtained commercially. Control and experimental animals were given food and water *ad libitum*. On day 13 of gestation (day of conception equal to day zero) the abdominal area of the experimental rats was exposed to 250 kVcp x rays. The total dose was 125 rads at a dose rate of 77.1 rads/minute. Both groups of rats were allowed to deliver their offspring and experiments were performed at 2 and 5 days postpartum. Two rats from each of six control litters and two rats from each of six x-irradiated litters (a total of 12 controls and 12 irradiated) were used on each day. The rats were injected intraperitoneally with ^{14}C-labeled phenylalanine and sacrificed 20 minutes later. The brains were excised and homogenized in a volume of distilled water equal to 12 times the brain weight. Aliquots (0.2 ml) of each of the homogenates were precipitated with 4 ml of 10 percent trichloroacetic acid (TCA) and centrifuged. The precipitate was washed three times with 4 ml of 10 percent TCA and the supernatants (acid soluble fraction) were combined (the second wash was carried out at 90°C for 30 min). One ml of the combined supernatant was counted in a liquid scintillation counter to determine the amount of ^{14}C-phenylalanine in the free amino acid pool. The precipitates were washed with 5 ml alcohol-ether (3:1 v/v) followed by an ether wash and dried. The dried precipitates were dissolved in 1 ml concentrated formic acid and $\frac{1}{2}$ ml portions were counted in a liquid scintillation counter to determine the amount of ^{14}C-phenylalanine in protein.

Aliquots (0.5 ml) of the brain homogenate were also taken for the determination of protein by the Lowry method (8).

Results and Discussion

The results for control and x-irradiated animals at 2 and 5 days postpartum are summarized in Table 1. The brain weights of x-irradiated animals are approximately 39 percent lower than controls at both 2 and 5 days postpartum ($P < .05$). This provides further confirmation of the well documented stunting effect of radiation on the fetus. Also, the amount of protein per mg tissue is greater (approximately seven percent) in the x-irradiated animals than in controls at 2 days of age ($P < .05$). However, no significant difference in this parameter was observed at 5 days postpartum. Although the amount of protein per mg tissue increased 2 days postpartum following x irradiation in utero, the brain weight of irradiated animals decreased. The net effect is a 35 percent decrease in the amount of protein in the whole brain of irradiated animals 2 days postpartum. It will be further shown that x irradiation is associated with depressed incorporation of ^{14}C-phenylalanine into protein. Therefore it is probable that the increased protein per mg tissue is an artifact that reflects a decrease either in water or some other significant constituent of brain tissue.

To determine the amount of amino acid entering the brain, calculations were made based on the amount of ^{14}C-phenylalanine which had been injected into the animals. Table 1 gives values for %I.D./P+S, where %I.D. = percent injected dose of ^{14}C-phenylalanine, P = dpm ^{14}C-phenylalanine per mg protein, and S = dpm in acid soluble fraction associated with one mg protein. Thus %I.D./P+S is the percent of the injected dose which entered the brain and was available for incorporation per milligram protein. This value was significantly increased by x irradiation in animals of both ages ($P < .05$). The increase was approximately 21 percent in the 2-day-old and about 38 percent in the 5-day-old animals. The percent injected dose which entered the brain per mg tissue was also significantly increased by irradiation ($P < .05$). The increase was approximately 27 percent in the 2-day-old and about 38 percent in the 5-day-old animals.

These results indicate that a greater amount of amino acid entered the brains of x-irradiated animals and suggest that the permeability of cells constituting the blood-brain barrier is increased by in utero x irradiation.

Table 1 also presents values for the percent of available labeled phenylalanine incorporated into protein.

The values for percent incorporation given in Table 1 were calculated as follows:

$$\frac{P}{P+S} \times 100 = \%\ \text{Incorporation}$$

TABLE 1. BRAIN WEIGHT AND AMINO ACID INCORPORATION IN FETALLY IRRADIATED RATS[a]

	No. of Animals	Brain Wt., mg	$\frac{\text{mg Protein}}{\text{mg Tissue}}$	$\frac{\text{\% I.D.}}{\text{P + S}}$[b]	$\frac{\text{\% I.D.} \times 10^3}{\text{mg Tissue}}$	% Incorp.[c]
			Two Days Postpartum			
Controls	12	314 ± 10	0.0587 ± 0.0008	0.0863 ± 0.0046	5.07 ± 0.29	35.0 ± 1.4
Irradiated	12	193 ± 5	0.0625 ± 0.0013	0.105 ± 0.007	6.43 ± 0.43	23.4 ± 2.5
			Five Days Postpartum			
Controls	12	514 ± 20	0.0602 ± 0.0013	0.0586 ± 0.0057	3.40 ± 0.26	40.8 ± 2.3[d]
Irradiated	12	316 ± 13	0.0589 ± 0.0011	0.0811 ± 0.0077	4.73 ± 0.43	31.1 ± 2.3

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a. All differences between control and irradiated animals were statistically significant ($P < 0.05$) with the exception of mg protein/mg tissue at 5 days postpartum.

b. % I.D./P + S: % I.D. = percent injected dose of ^{14}C-phenylalanine
P = dpm ^{14}C-phenylalanine/mg protein
S = dpm ^{14}C-phenylalanine in acid soluble fraction associated with 1 mg of protein
P + S = approximate amount of ^{14}C-phenylalanine available for incorporation

c. % Incorp. = percent incorporation of ^{14}C-phenylalanine = $\frac{P}{P + S} \times 100$.

d. Mean value based on 10 observations.

The percent of available ^{14}C-phenylalanine incorporated into protein (% incorp.) is significantly depressed in irradiated animals at both 2 and 5 days ($P < .05$). The decrease in incorporation was approximately 33 percent in the 2-day-old and about 24 percent in the 5-day-old animals as compared with controls.

In spite of the fact that more amino acid entered the brain tissue, less amino acid was incorporated into protein in the x-irradiated animals. If the size of the amino acid pool does not increase following x irradiation, then the data reported here indicate that protein synthesis in the experimental animals is depressed.

Summary

Pregnant rats were x irradiated on day 13 of gestation (125 rads) and amino acid incorporation into brain protein was measured in the offspring at 2 and 5 days after birth. There is a significant decrease in incorporation of labeled amino acid into protein following irradiation. There is also a significant increase in the amount of amino acid entering the brain. These results suggest that *in utero* x irradiation depresses brain protein synthesis and causes some injury to the blood-brain barrier.

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PATHOLOGIC STUDIES SECTION

Dr. C. David Lytle, Chief

The research conducted by the Pathologic Studies Section investigates biological systems at several levels of complexity following radiation of several different types, ionizing and nonionizing.

Viruses and mammalian cells are being studied with regard to responses following ultraviolet light or x-ray exposure. Radiation caused tumor cells in culture are being characterized. Biological effects of microwaves are being investigated with rats (see Dosimetric Studies Section) and dogs as experimental subjects. X-ray studies on animals are directed at tumorigenesis, cataracts, and age dependence of sensitivity.

The major findings of this section over the past year include the following:

(1) Mouse cells after exposure to ultraviolet light are more susceptible to transformation by a tumor virus.

(2) An irradiated mammalian virus is more infectious in monkey cells that have been exposed to small doses of ultraviolet light.

(3) Normal human cells are capable of repairing irradiated virus.

(4) Low-energy (TV-type) x rays cause cataracts as easily as higher energy x rays.

(5) Immune suppressive drugs are not synergistic with x irradiation for causing rat mammary tumors.

(6) Ultrastructural examinations of bone marrow from strontium-89 fed cats reveal degenerative changes.

A. ENHANCEMENT OF VIRAL TRANSFORMATION BY ULTRAVIOLET LIGHT

C. D. Lytle, K. B. Hellman, and N. C. Telles

Many factors can affect the frequency with which an oncogenic virus can cause in vitro transformation of mammalian cells. X radiation has been shown to make cells more susceptible to viral transformation. It is of interest to determine if ultraviolet (UV) light, a carcinogenic radiation of lower energy, can produce a similar effect. This report demonstrates that UV-irradiation of mouse cells enhances their transformation by the DNA tumor virus, SV40.

The materials and procedures used were essentially those reported by Pollock and Todaro (1). The radiation source was a germicidal lamp. The cells were irradiated and infected as confluent cultures. Twenty to twenty-four hours after being irradiated the cells were subcultured at several dilutions for transformation and colony-formation assays. The cells were fixed and stained at 18 days. The transformed colonies were counted on the same plates as the total surviving colonies and were identified by their multi-layered structure, characteristic of transformed cells in vitro.

The survival curve in Figure 1 shows that the colony-forming ability of the infected cells decreased with increasing UV dose with a D_0 of about 66 erg/mm^2 and an extrapolation number of about 10. A control experiment using uninfected cells gave a similar survival curve. The number of transformed colonies decreased with irradiation (Table 1), although more slowly than the colony-forming ability. These data indicate that the fraction of surviving colonies that are transformed increases with UV dose. The uninfected irradiated controls had no identifiable transformed colonies.

TABLE 1. SURVIVAL OF TRANSFORMED AND TOTAL COLONY-FORMING ABILITY AFTER UV-IRRADIATION

UV Dose erg/mm^2	Total Colony-Forming Units	Transformed Colony Units
0	2.7×10^5	8.1×10^3
136	1.3×10^5	4.4×10^3
180	9.2×10^4	7.4×10^3
272	2.0×10^4	1.0×10^3
408	8.0×10^3	1.6×10^3
450	1.6×10^3	2.6×10^2
544	1.0×10^3	1.6×10^2

The relation of the transformation frequency of the survivors to dose is shown in Figure 2. The UV dose necessary to enhance the transformation by a factor of e^1 (analogous to D_0 for inactivation) was about 310 erg/mm^2. Thus the UV target for transformation enhancement appeared to be considerably smaller than that for inactivation of colony-forming ability for these cells. This is different from the results obtained with x rays. Using essentially the same methods, the same virus, and very similar cells, Pollock and Todaro (1) found nearly equal sensitivities to lethality and transformation enhancement for 250 kVp x rays. Thus x rays are more effective per lethal hit at transformation enhancement than UV.

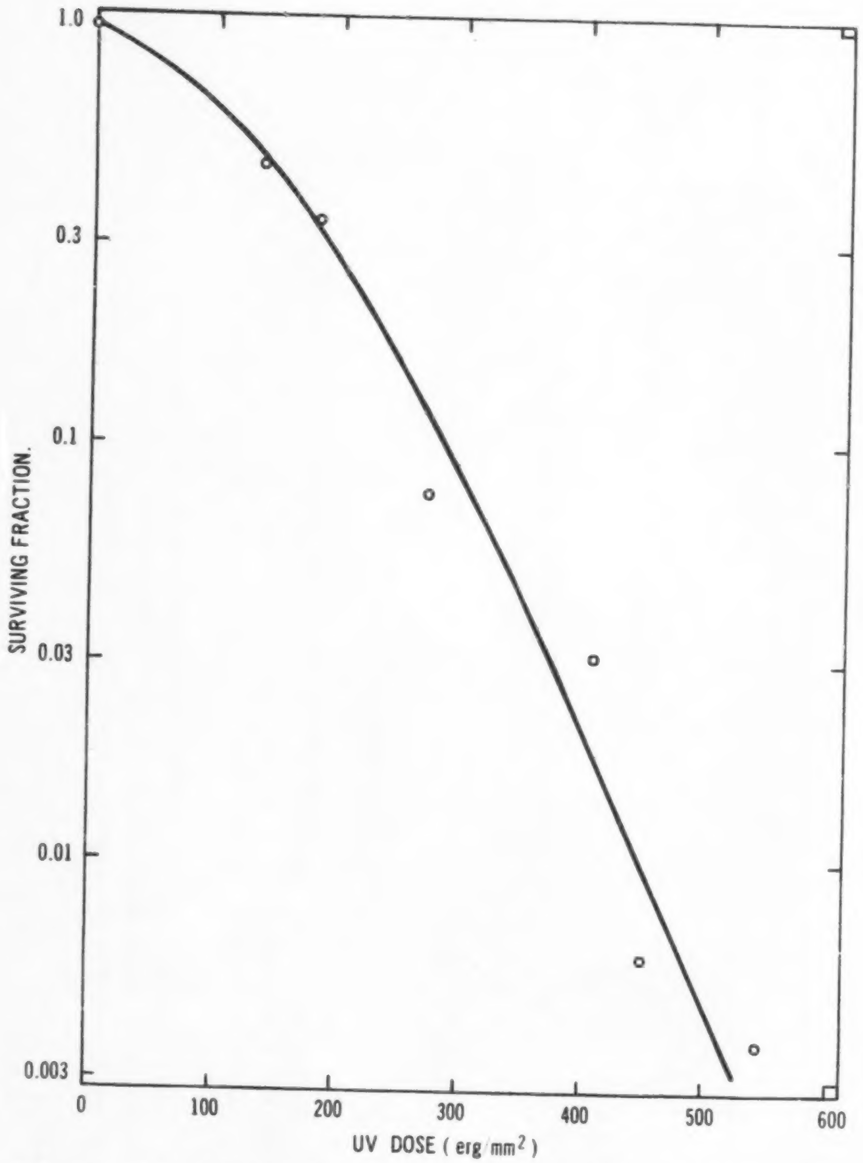

Figure 1. Survival of Colony-Forming Ability of SV40-Infected Balb/3T3 when Subcultured 20 Hours After Irradiation.

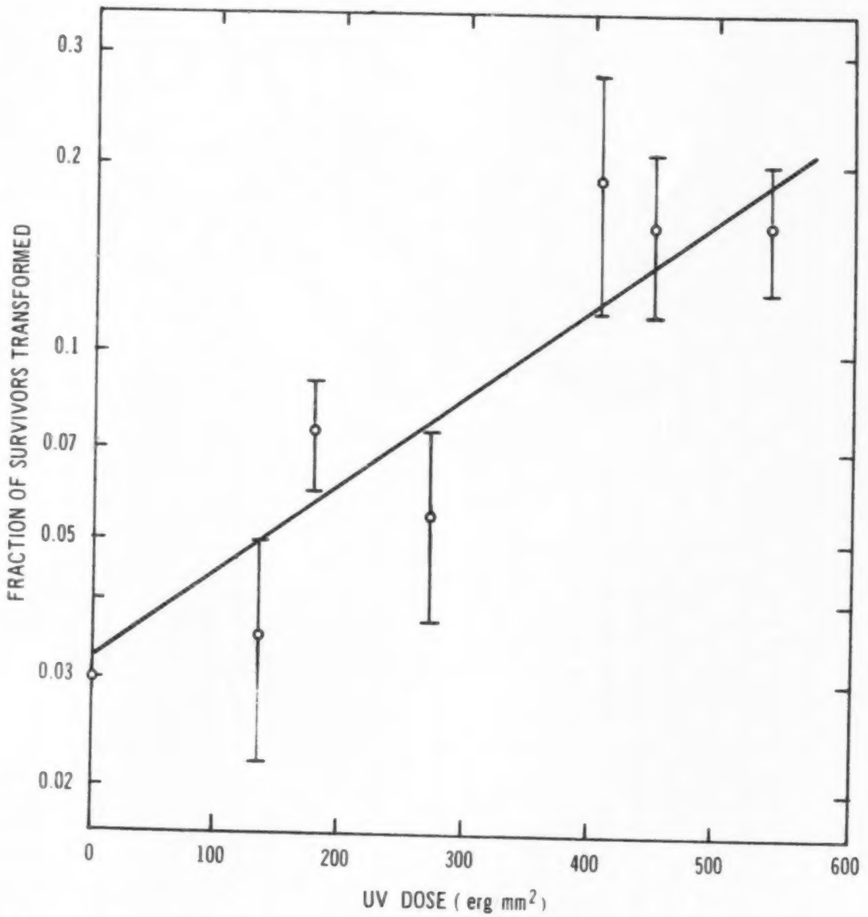

Figure 2. Enhancement of Transformation Frequency.

The question arises whether the mechanism for enhancement of transformation operates by selective killing of cells that are less susceptible to transformation, or by an actual increase in transformation susceptibility in most cells. The latter alternative is suggested when the absolute number of transformants increases with radiation exposure. This was not obtained in these experiments, although an absolute increase was found in this system when x rays were used. Thus, although x rays apparently increase the transformation susceptibility of cells, it is not known if this is the case for UV irradiated Balb/3T3 cells. There is evidence that UV can enhance transformation susceptibility of hamster embryo cells. For this reason, we believe that at least part of the enhancement by UV could be an increase in transformability.

The differences between the effectiveness of x rays and that of UV probably lie in the types of physical-chemical damage and the extents and types of repair that occur. X rays are known to cause repairable strand breaks in the DNA of mammalian cells. Thus, if, as current hypotheses suggest, permanent viral transformation requires integration of viral DNA into cellular DNA and if DNA strand breaks are necessary for integration, the x-ray damage can directly aid transformation by providing DNA strand breaks. UV, on the other hand, causes base damage but unmeasurable strand breakage at the doses used here. Therefore, the UV damage itself may not directly enhance transformation. However, since DNA strand breaks are created during the repair of UV damage, the presence of viral DNA at the repair site might lead to integration as an indirect result of UV damage.

Although viruses have been implicated in many animal tumors, their role in UV carcinogenesis remains to be demonstrated. The experiments reported here suggest that UV is capable of promoting viral tumorigenesis.

Reference

Pollock, E.J., and G.J. Todaro. 1968. Radiation enhancement of SV40 transformation in 3T3 and human cells. Nature 219:520-521.

B. DECREASED HOST CELL REACTIVATION OF IRRADIATED SV40 VIRUS IN XERODERMA PIGMENTOSUM

S. A. Aaronson[1] and C. D. Lytle

Host cell reactivation (HCR) is manifested as the lowered survival of ultraviolet irradiated viruses in radiation-sensitive mutant host cells. Mammalian cells possess repair systems. In xeroderma pigmentosum, a genetic disease associated with unusually high sensitivity to sunlight and a greatly increased susceptibility to carcinomas and melanomas of the skin, the capacity of fibroblast cells for repair is much lower than that of normal fibroblasts. Recent evidence suggests that xeroderma pigmentosum cells are defective in the first reaction of the DNA repair mechanism. Previous investigations have raised the possibility of a host cell reactivation system in mammalian cells. We have shown directly that normal human cells have HCR capacity by comparing the ultraviolet sensitivities of two viral functions of the DNA tumor virus SV40--transformation and T-antigen induction--in normal and xeroderma pigmentosum cells.

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[1] National Cancer Institute

The SV40 virus induces permanent alterations in the morphology and growth properties of several mammalian species in tissue culture including human cells. Transformants can be recognized by their ability to form dense, piled-up colonies against the background of normal contact-inhibited cells. This lack of normal growth restraint induced by the virus provides the basis for the quantitative assay of transformation. Two to three weeks after infection with SV40, densely growing colonies of epithelioid cells could be recognized as early transformed colonies. The transformation frequency was expressed as the total number of transformed colonies obtained per total number of cells inoculated. The transformation frequency with the control SV40 was approximately 0.25 percent for each cell strain. Thus, if the ultraviolet repair enzyme or enzymes deficient in xeroderma pigmentosum cells were necessary for the process of transformation, their presence at markedly lower levels still permitted a normal transformation frequency in the xeroderma pigmentosum cells. The ultraviolet survival curves for transformation are shown in Figure 1. The data are adequately described by single hit, exponentially decreasing functions. The ultraviolet survival curve of transformation in both types of xeroderma pigmentosum cells was considerably steeper than that of the normal cell strain, M. G. The slope for the most ultraviolet sensitive cell strain (XP-1) was about eight times greater than the slope for the normal fibroblasts.

SV40 T antigen is known to appear in acutely infected cells and to persist in transformed cells. It can be localized in the cell nucleus by an indirect fluorescent antibody test. With infection by normal SV40, the number of human cells synthesizing T antigen during the acute infection is maximal at 72 hours. The fraction of normal and xeroderma pigmentosum cells containing the antigen at this time was scored. The ultraviolet survival curves are compared in Figure 2. As with transformation, the ultraviolet sensitivity of T-antigen induction was greater in the two radiation-sensitive human cells. The slopes for XP-1 and XP-2 were calculated to be 9.0 and 5.0 times greater than normal, respectively.

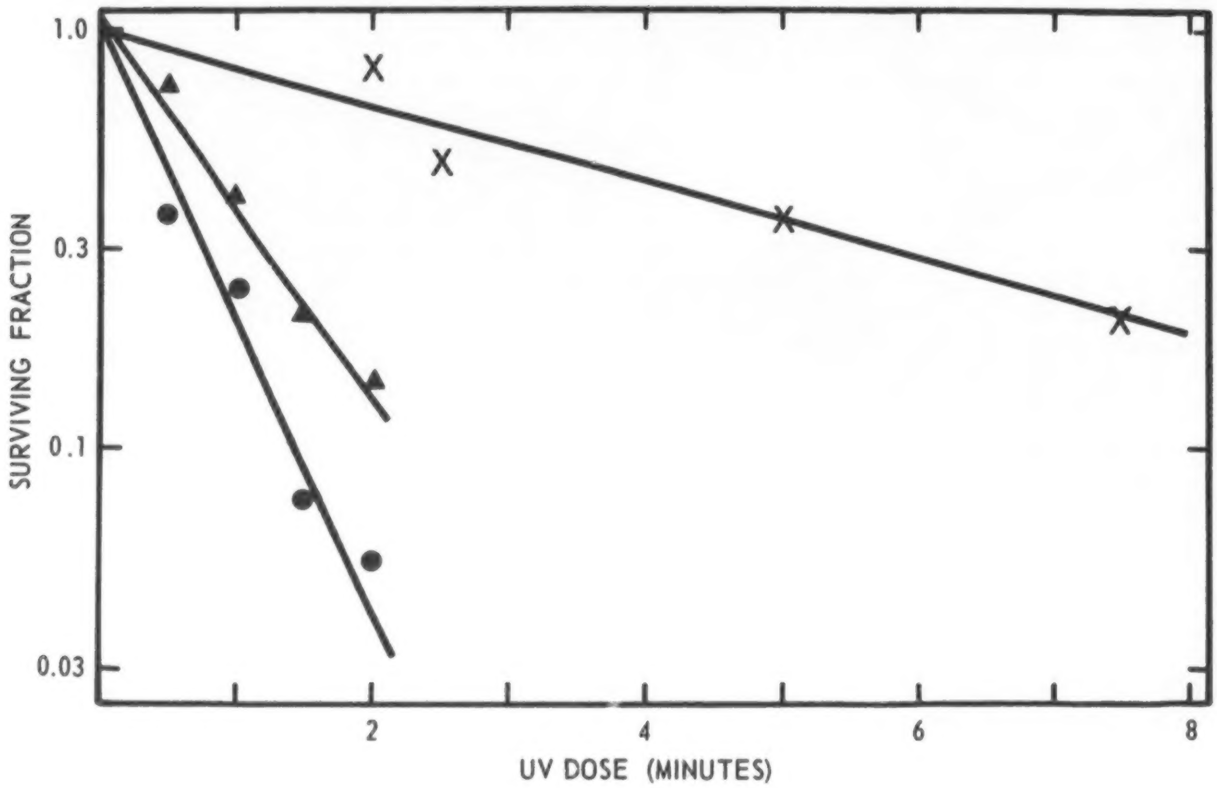

Figure 1. Ultraviolet Survival Curves of SV40 Transformation in Normal and Xeroderma Pigmentosum Cells. XP-1 ●; XP-2 ▲; M.G. ×.

Our findings are consistent with the hypothesis that xeroderma pigmentosum cells have little or no ability to reactivate ultraviolet-damaged SV40. The kinetics of T-antigen induction in normal human and mouse cells are delayed for ultraviolet-irradiated virus, and we therefore studied the kinetics of T-antigen formation with ultraviolet-damaged SV40 in the xeroderma pigmentosum cells. Figure 3 shows the percentages of T-antigen positive cells at different times after infection of normal and xeroderma pigmentosum cell strains by the control SV40 and by the ultraviolet-irradiated SV40. The T-antigen levels for control virus were 5-7 percent in each cell strain after 3 days and thereafter declined at similar rates. There was a definite delay in the induction of T antigen in the normal cells by the ultraviolet-irradiated virus. With the xeroderma pigmentosum cells, also, the timing of the induction of T antigen by ultraviolet-irradiated SV40 was altered. The time course of T-antigen appearance was similar in xeroderma pigmentosum and normal cells, although the absolute T-antigen levels were much higher in the

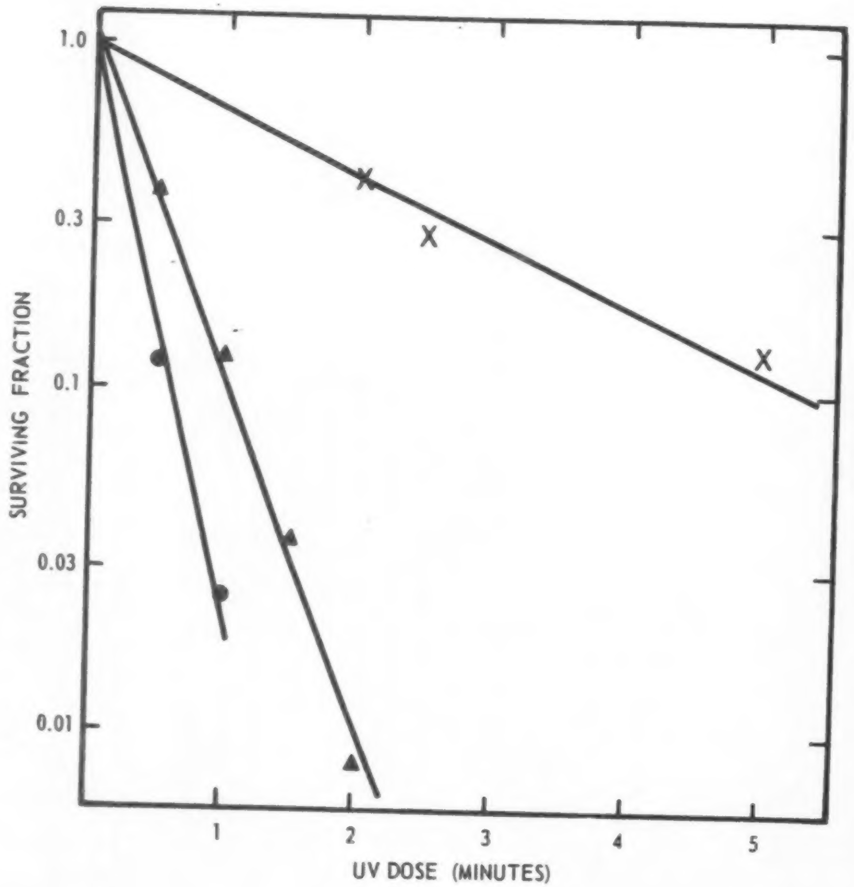

Figure 2. Ultraviolet Survival Curves of SV40 T Antigen in Normal and in Xeroderma Pigmentosum Cells. XP-1 ●; XP-2 ▲; M.G. ×.

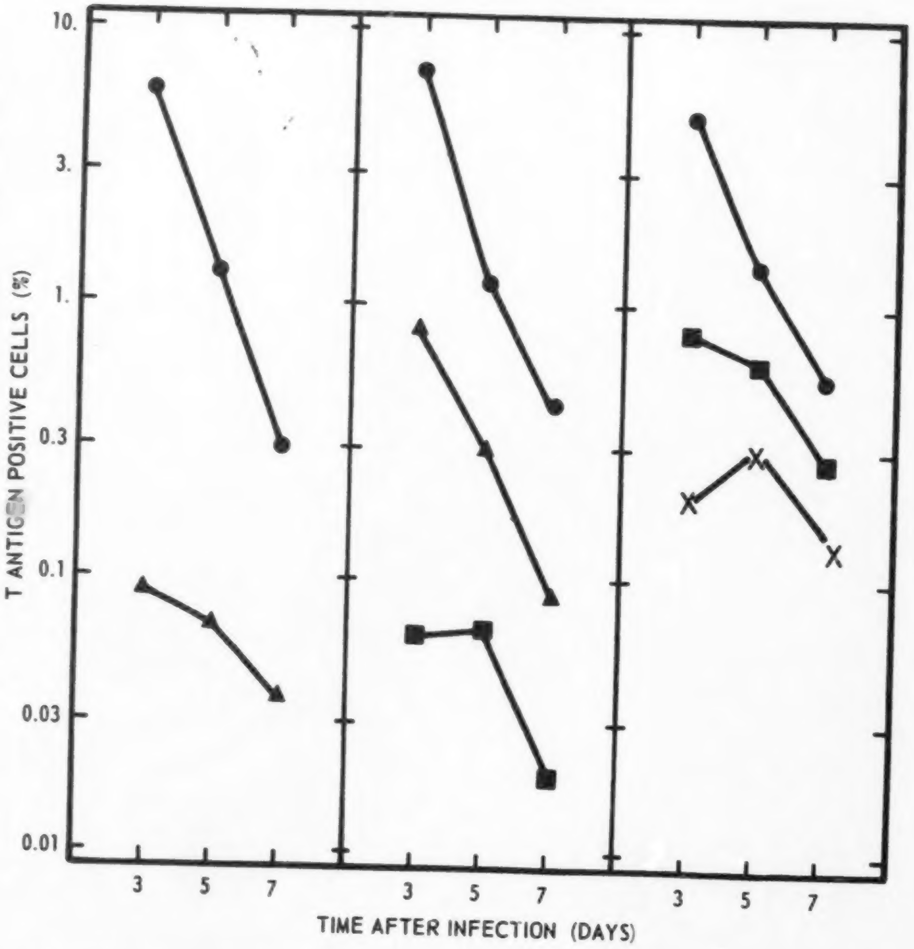

Figure 3. T Antigen Levels Measured at Different Times after Infection by Control and Ultraviolet-Irradiated SV40. Ultraviolet doses: ●, 0 min; ▲, 1 min; ■, 2 min; ×, 5 min. Left, XP-1; middle, XP-2; right, M.G.

latter. This suggests that although the probability of host cell reactivation of damaged virus in the xeroderma pigmentosum cells was much lower than in normal cells, if reactivation did occur, it did so with a time course similar to that seen in the normal cells.

We have compared the survival of two functions of the tumor virus SV40 in normal and xeroderma pigmentosum cells at identical virus multiplicities. The results clearly showed a markedly lower survival of both transformation and T-antigen functions in the xeroderma pigmentosum cells. By analogy with studies in bacteria, this host cell effect on ultraviolet-damaged virus is most consistent with the hypothesis that ultraviolet-damaged SV40 is repaired by enzymes in the normal human cell that are missing or at much lower levels in xeroderma pigmentosum cells. The results also indicate that enzymes present in normal human cells can reactivate an ultraviolet-irradiated DNA tumor virus. The data obtained with strains XP-1 and M. G. indicate that the normal cell can have 8-9 times more HCR capacity than the repair-deficient cell.

Strain XP-1 produced steeper survival curves than XP-2 for both T antigen and transformation functions. That consistent differences were found in the ability of the two xeroderma pigmentosum strains to "reactivate" damaged SV40 implies that these cells have relative and not absolute defects in their DNA repair capacity. The repair replication ability of strain XP-1 was tested by Dr. J. Cleaver (personal communication) and found to be about 5 percent of the normal using his assay methods. Our results showing about 12 percent of the normal HCR in XP-1 indicate that there may be a close correlation between the capacities for repair replication and HCR in human cells.

C. HOST CELL REACTIVATION IN MAMMALIAN CELLS

C. D. Lytle

It has recently been shown that host cell reactivation (HCR) of UV-irradiated mammalian viruses can occur. Skin fibroblasts from patients having the genetic disease xeroderma pigmentosum apparently have much lower capacity for reactivating UV-damaged SV40 virus than skin cells from normal individuals, correlating well with their respective capacities for repair replication. Cells from several different species of mammals have been found to have different repair replication abilities. Together these studies suggest that different mammalian cells may have different HCR abilities. This report confirms the hypothesis using herpes simplex virus with cells of mouse, rat, monkey, and human origin.

Herpes simplex virus was used in these studies to infect cells of mouse, rat, monkey, and human origin. The virus suspensions were irradiated with a germicidal lamp. The standard herpes simplex plaque assay

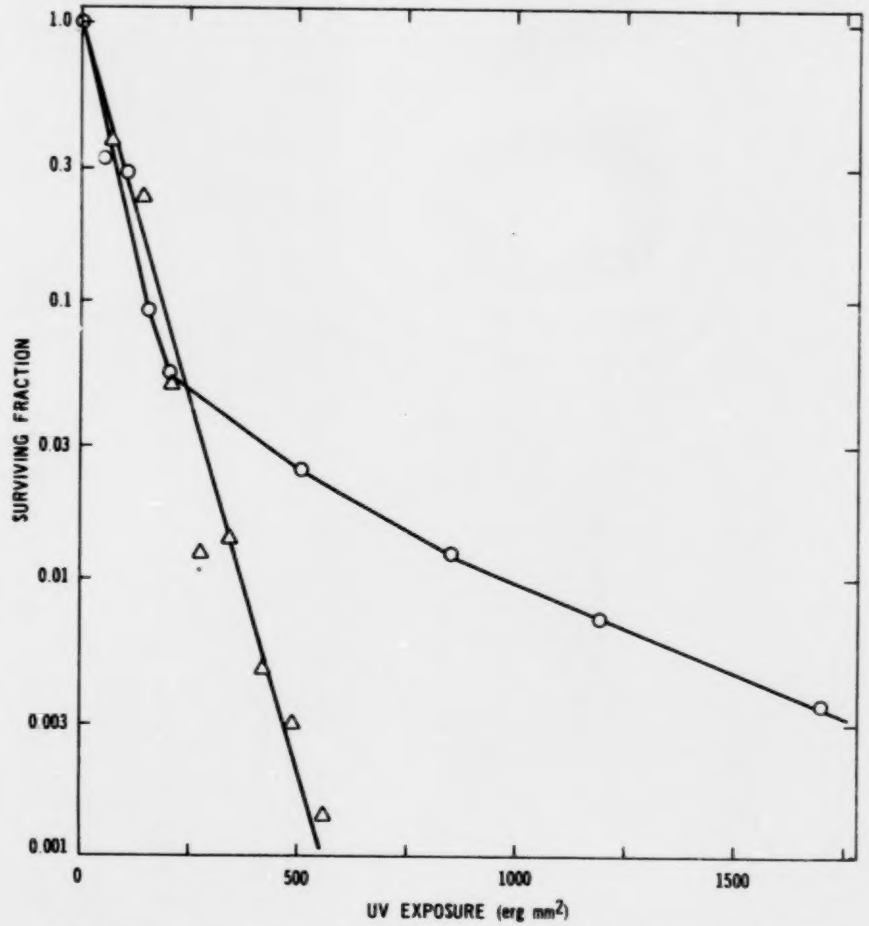

Figure 2. Survival Curves for UV-Irradiated Herpes Simplex Virus in Cells of Rodent Origin: Rat, RE (○); Mouse, Balb/3T3 (Δ).

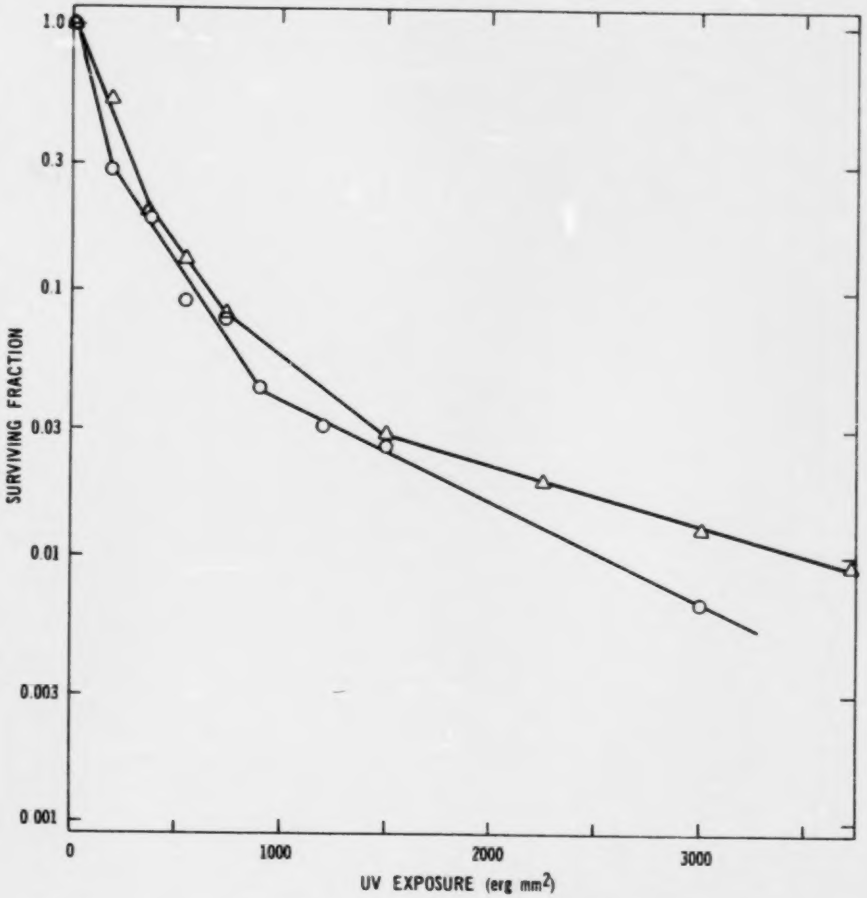

Figure 3. Survival Curves for UV-Irradiated Herpes Simplex Virus in Kidney Cells of the African Green Monkey: GMK (○), CV-1 (Δ).

Therefore the survival curves of UV-irradiated herpes simplex virus had one or two components in these mammalian cells. Table 1 shows the dose required for an average of one lethal hit, e^{-1} dose, for each component of the survival curves.

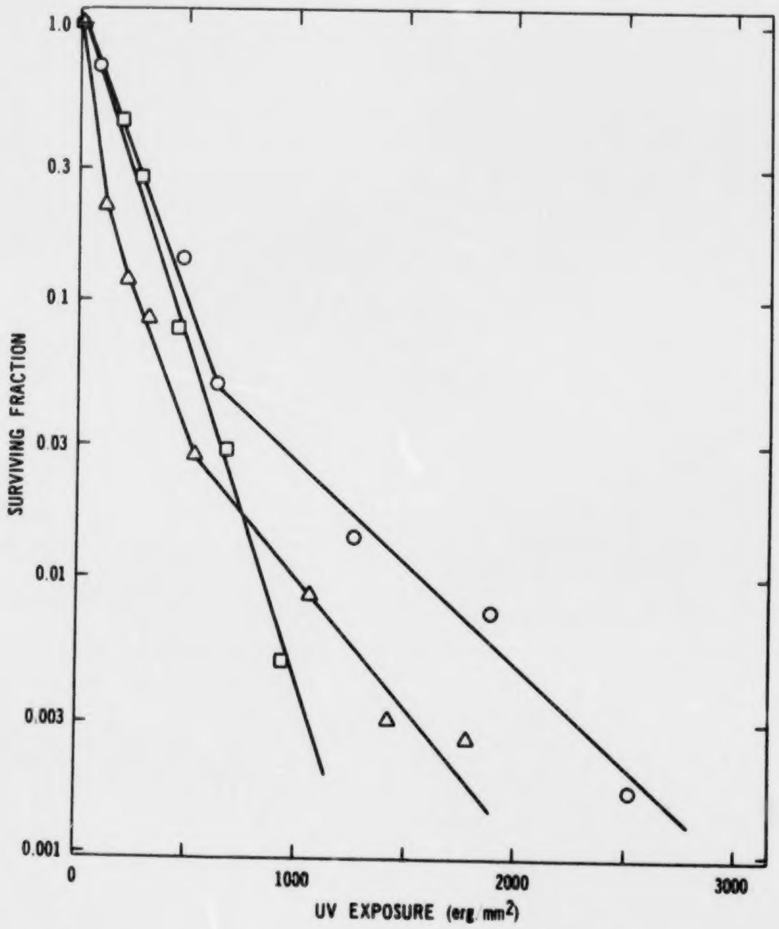

Figure 4. Survival Curves for UV-Irradiated Herpes Simplex Virus in Cells of Human Origin: HEp-2 (○), WI38 (Δ), TF (□).

The e^{-1} doses for the initial slopes (first components) have a range over a factor of about four (55 to 220 erg/mm^2). The viruses assayed in rodent cells are more UV sensitive (lower e^{-1} dose) than when assayed in monkey cells, while the apparent UV sensitivities in different human cells fell near both ends of the range. The e^{-1} doses for the final slopes (second components) have a range of similar magnitude (460 to 1900 erg/mm^2). For this component the viruses assayed in monkey cells were less sensitive than in the other cells. In this case the assays in human cells were at the more sensitive end of the sensitivity range. Among these data there is no apparent quantitative relation of sensitive component to resistant one.

TABLE 1. DOSES REQUIRED FOR ONE LETHAL HIT FOR
UV INACTIVATION OF HERPES SIMPLEX VIRUS ASSAYED ON DIFFERENT CELLS

Cell Strain	Origin of Cells	e^{-1} Dose for First Component, (erg/mm^2)	e^{-1} Dose for Second Component, (erg/mm^2)	% Infected Cells Represented by Second Component
RE	Rat	55	670	4-5
Balb/3T3	Mouse	80	-	-
GMK	Monkey	160	1150	9-10
CV-1	Monkey	220	1900	6-23
WI-38	Human	80	460	5-9
HEp-2	Human	165	600	10-20
TF	Human	175	-	-

The relative amounts of the infected cells expressing either of the two components can be determined from the survival curves. The extrapolation of the resistant component to zero dose gives the fraction of the total infected cells which are represented by this component. For those cells which demonstrated a resistant component, this component constituted less than 25 percent of the total infected cells (see Table 1).

Multiplicity reactivation has been found to account for resistant components in survival curves for certain other viruses. This phenomenon is produced when cells are infected with large numbers of damaged virus permitting the virus genomes to complement functional and genetic information. With multiplicity reactivation the number of plaques is not proportional to the virus concentration since more than one virus particle is needed for the complementation. Thus a one-to-one relation between plaques and virus concentration is proof that multiplicity reactivation is not occurring. Figure 5 shows that the number of plaques formed are proportional to the virus concentration in CV-1 cells for even the highest UV doses to the virus. Therefore, the resistant component of the survival curve in CV-1 cells cannot be explained by multiplicity reactivation.

Survival of UV-irradiated herpes simplex virus was assayed in CV-1 cells using medium containing caffeine, an inhibitor of bacterial host cell reactivation. The survival curves in Figure 6 show that the presence of caffeine lowered the survival of the resistant component.

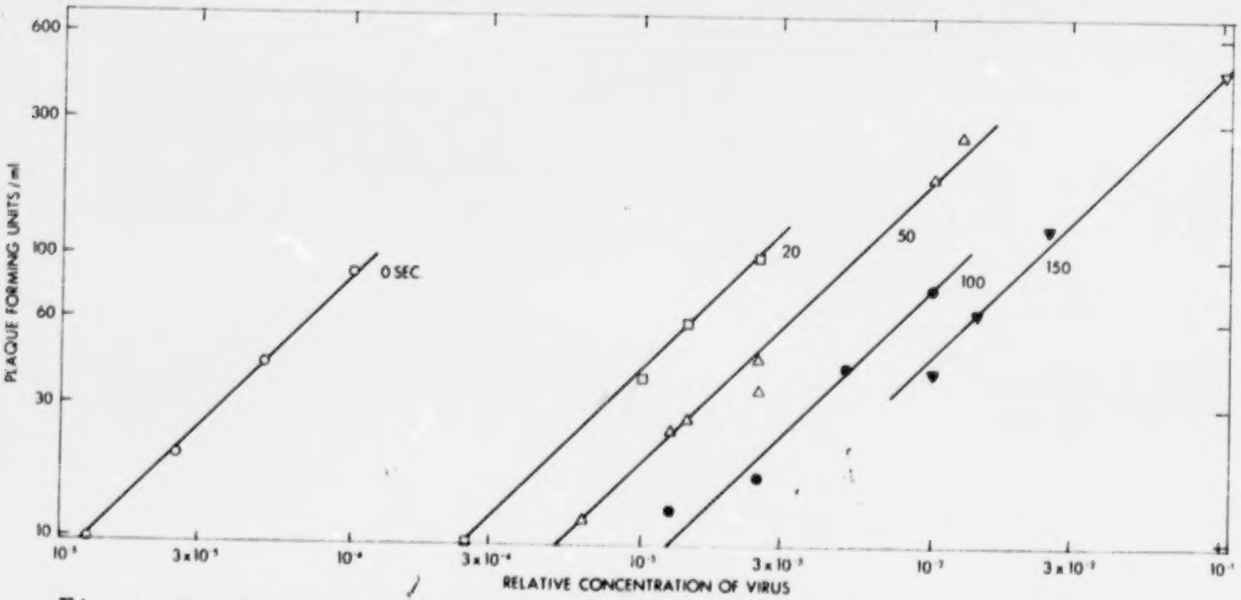

Figure 5. Demonstration of Linear Relation Between Number of Plaques Formed and Virus Concentration in Infecting Inoculum for UV-Irradiated Herpes Simplex Virus Assayed in CV-1 Cells. UV doses to the virus: 0 sec (○), 20 sec (□), 50 sec (△), 100 sec (●), 150 sec (▼).

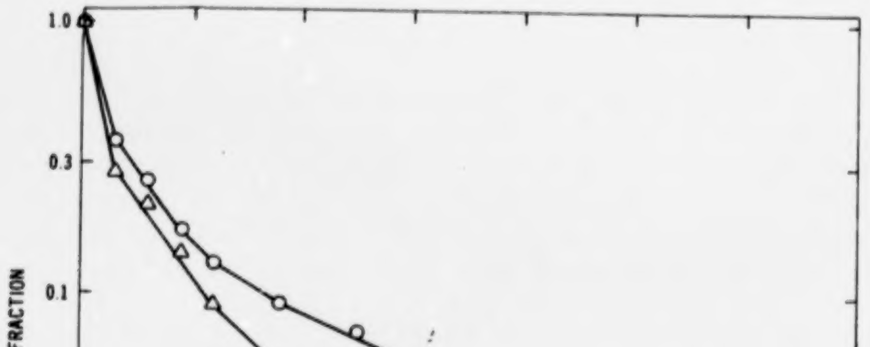

Figure 6. Survival Curves for UV-Irradiated Herpes Simplex Virus in CV-1 Cells in the Presence (△) and Absence (○) of 3 mM of Caffeine.

Survival curves for many UV-irradiated animal viruses are multicomponent even when the cells and viruses used are assumed to be homogeneous and multiplicity reactivation was not present. This study showed that most UV-survival curves for herpes simplex virus have two components in mammalian cells.

For bacteriophage it has been thought that HCR was demonstrated only in the second (resistant) component for two component survival curves. This reasoning has been extrapolated to animal virus survival. In this report it was shown that for herpes simplex virus in CV-1 cells the second component in UV-survival curves is reduced by caffeine, a property similar to the HCR in bacteria. In addition, enhancement of survival by UV-irradiating the assay cultures (ultraviolet reactivation - demonstrable for bacteria only in HCR positive strains) apparently occurs only for the second component (1). Thus, the host cell effect in the resistant component of herpes virus survival appears to be analogous to bacterial HCR. The differences in slopes of the second components of the survival curves among the mammalian cells may be a result of different capacities for HCR in the different cells.

The data presented here indicate that some form of host cell effect is also present in the first, more UV-sensitive component. The magnitude of the differences in this "HCR" effect (up to a factor of 4) is large enough to be easily measured and approaches the differences found for bacteriophage assayed in HCR positive and HCR negative bacterial strains (factors of 4 to 8).

The recovery processes responsible for the two components may be different, since the slopes of the components seem to vary independently from one cell type to another. Therefore the evidence presented here may indicate the presence of two host cell reactivation processes: One which provides recovery from low doses of UV irradiation (first component), and the other from high doses (second component).

Reference

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D. ESTABLISHMENT OF A CROSS-INDEXING FILING SYSTEM FOR PATHOLOGIC DIAGNOSES OF ANIMALS USED IN EXPERIMENTS AT THE DIVISION OF BIOLOGICAL EFFECTS

Jerrold M. Ward and Arthur H. McTighe

In order to have the means to retrieve information from the slide and card files of the histopathology laboratory, a cross-indexing filing system was initiated. Since 1968, slides have been prepared from nearly 5,000 animals (Osborne-Mendel and Long-Evans rats, Chinese hamsters, cats, and a few other species). The card and slide files were reviewed and information was obtained from them and put into the cross-indexing system. The most common diagnoses and numbers of slides of each are given below for two species.

Rats (Primarily Long-Evans)

Chronic murine pneumonia	100	Thyroid tumors	
Nephritis	66	adenoma	10
Pleuritis	32	carcinoma	41
Bronchopneumonia	25	Lymphosarcoma	39
Hydronephrosis	13	Hepatoma	17
Metritis	11	Pulmonary carcinoma	12
Focal liver necrosis	8	Female genital tract tumors	11
Nodular hyperplasia of liver	8	Fibrosarcoma	8
Peritonitis	7	Squamous cell carcinoma	6
Meningitis	7	Renal carcinoma	5
Mammary tumors		Brain tumors	4
fibroadenoma	95	Ovarian carcinoma	3
cystadenoma	39	Pancreatic tumors	3
malignant tumors	23	Intestinal tumors	2
fibroma	4	Granulocytic leukemia	2
Pituitary adenoma	68		

Cats (From the Toxicologic Studies Section)

Pneumonia	10	Pyelonephritis	2
Enteritis	6	Lymphosarcoma	1
Toxoplasmosis	5	Feline herpesvirus disease	1
Cystitis	5	Hepatic abscesses	1
Hemorrhagic diatheses	2	Encephalitis	1
Renal cysts	2	Hepatopathy	1

E. ULTRASTRUCTURAL STUDIES OF CAT TISSUES

Jerrold M. Ward, Gwendolyn Harley, and Gordon Wharran

Bone marrow, kidney, and salivary glands of normal cats and cats fed strontium 89 are being studied ultrastructually to detect the presence of feline leukemia virus and to characterize normal and abnormal cells and tissues.

To date, bone marrow from four cats with strontium-89 induced hemorrhagic diatheses have been studied. Besides a change in the Myeloid: Erythroid (M:E) ratio, increased numbers of plasma cells have been observed in bone marrows of two cats. Degenerative changes in mitochondria of many plasma cells and occasionally of other cells, have been found in one cat (Figures 1, 2, 3). These changes have been reported previously in cells from animals exposed to a variety of types of ionizing radiation.

Normal bone marrow from three cats, normal kidneys from three cats, one salivary gland, and neoplastic tissue from the cat with spontaneous lymphosarcoma in the colony have also been examined. Viral particles have not been seen in any normal or abnormal tissues studied.

Continuing ultrastructural studies will be aimed at determining if strontium-89 induced hematologic diseases and leukemias in cats result from release of a latent leukemia virus.

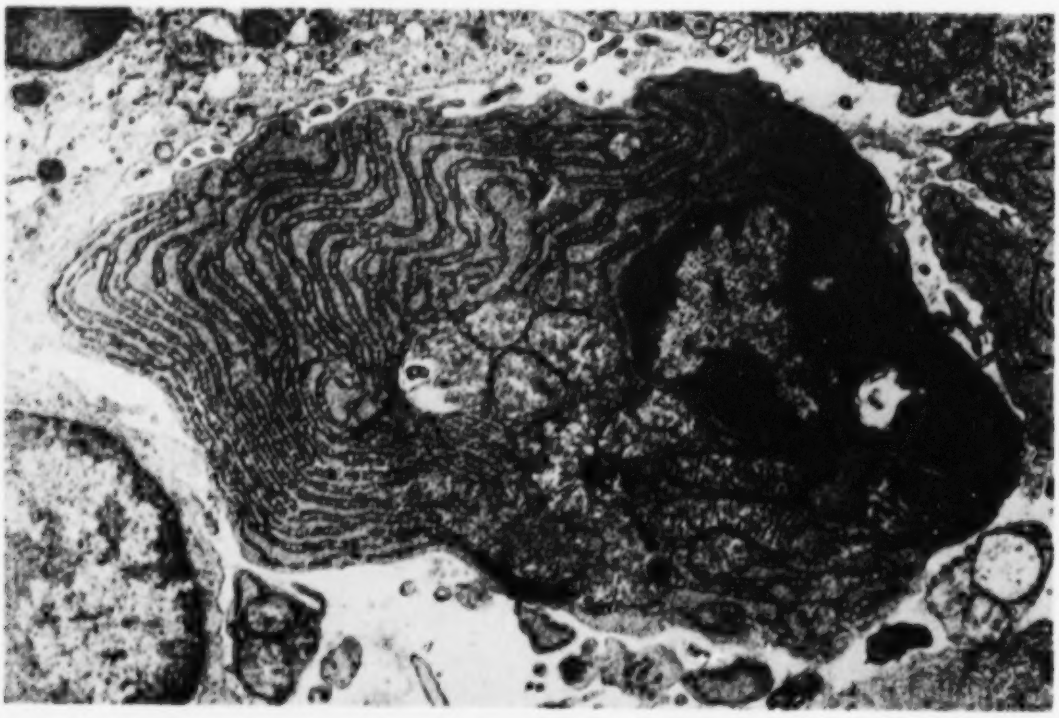

Figure 1. Electron Micrograph of Plasma Cell in Bone Marrow of Cat with Strontium-89 Induced Hemorrhagic Diathesis. Arrows point to degenerative changes in three mitochondria. M denotes normal mitochondrion.

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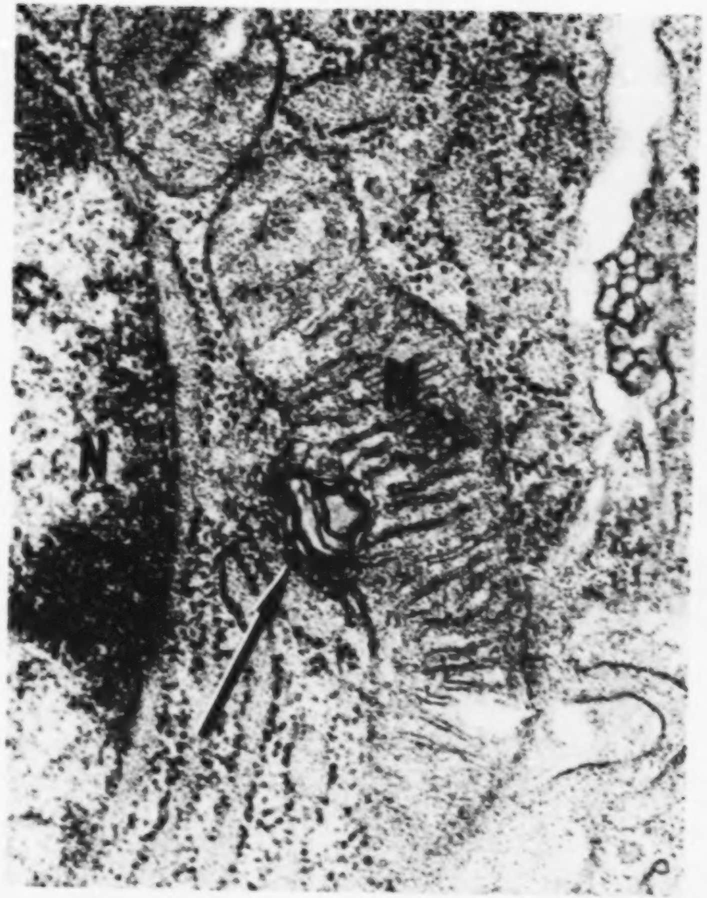

Figure 2. Early Changes in the Mitochondrial Membranes (arrow). Mitochondrion (M), Nucleus (N).

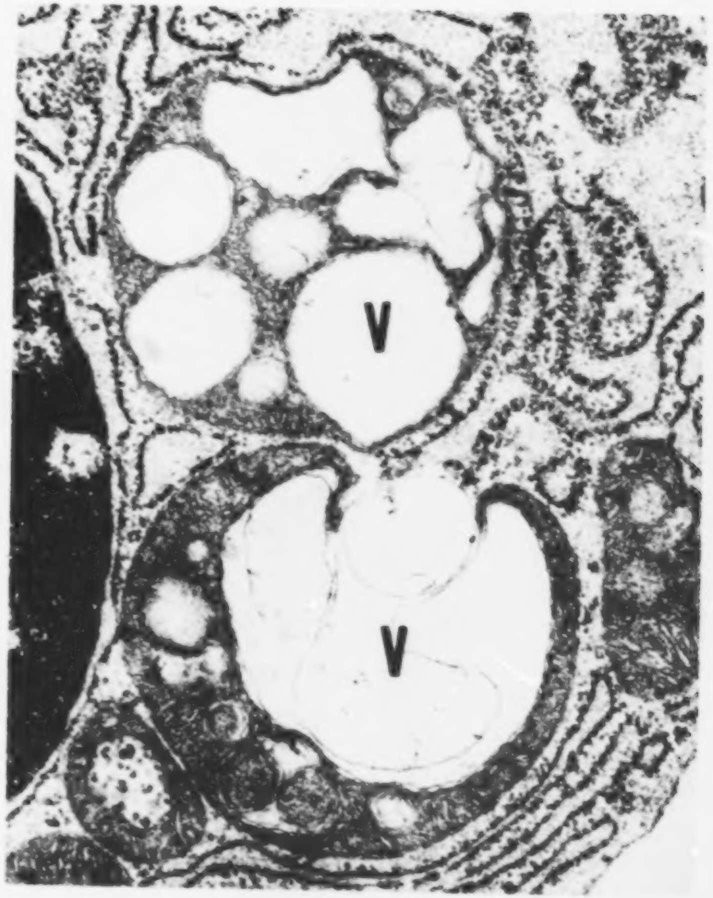

Figure 3. Advanced Degenerative Changes in Two Mitochondria. Large vacuoles (V) are seen in the greatly enlarged mitochondria.

F. ULTRAVIOLET LIGHT ENHANCED REACTIVATION OF A MAMMALIAN VIRUS

L. E. Bockstahler and C. D. Lytle

It is known that there is an increase in the survival of certain UV-irradiated bacterial viruses when they infect lightly UV-irradiated host cell bacteria (1). This effect is called UV-reactivation (UVR). The purpose of this investigation was to determine whether or not a similar effect might occur for a human virus - mammalian cell system. It was found that indeed the survival of UV-irradiated herpes simplex virus, a common human virus, is enhanced after inoculation onto lightly UV-irradiated CV-1 cells, a permanent line of monkey kidney cells (2).

Confluent monolayers of CV-1 cells grown in petri dishes were lightly UV-irradiated at various doses. UV-irradiated herpes simplex virus was assayed on these cells (Figure 1a). UVR was not apparent for virus irradiated with low doses (15 sec. or less at 34 erg/mm /sec.). However, virus irradiated with higher doses exhibited UVR. In order to better characterize the UVR effect, the experiment was repeated for virus irradiated for 60 and 120 sec. and more data were taken for low doses to the cells (Figure 1b). These two curves clearly demonstrate UVR. The reason the shapes of these curves are not quantitatively the same as in Figure 1a has not yet been determined and may be due to a parameter of the cell system such as differences in cell ages at time of assay between the two experiments.

These results show that UVR exists in a mammalian virus-cell system and suggest that UVR may be a general phenomenon. Doses to the cells necessary for demonstrating UVR were an order of magnitude lower than those previously shown for bacterial virus - *E. coli* systems. The amount of enhancement of survival by UVR was approximately the same in both systems.

Demonstration of UVR in a mammalian virus-cell system is of interest since both viruses and humans are subjected to UV irradiation (sunlight, use of UV light for sterilization purposes, for example). Enhancement of irradiated virus survival following host cell irradiation may occur for other types of radiation and is presently under investigation.

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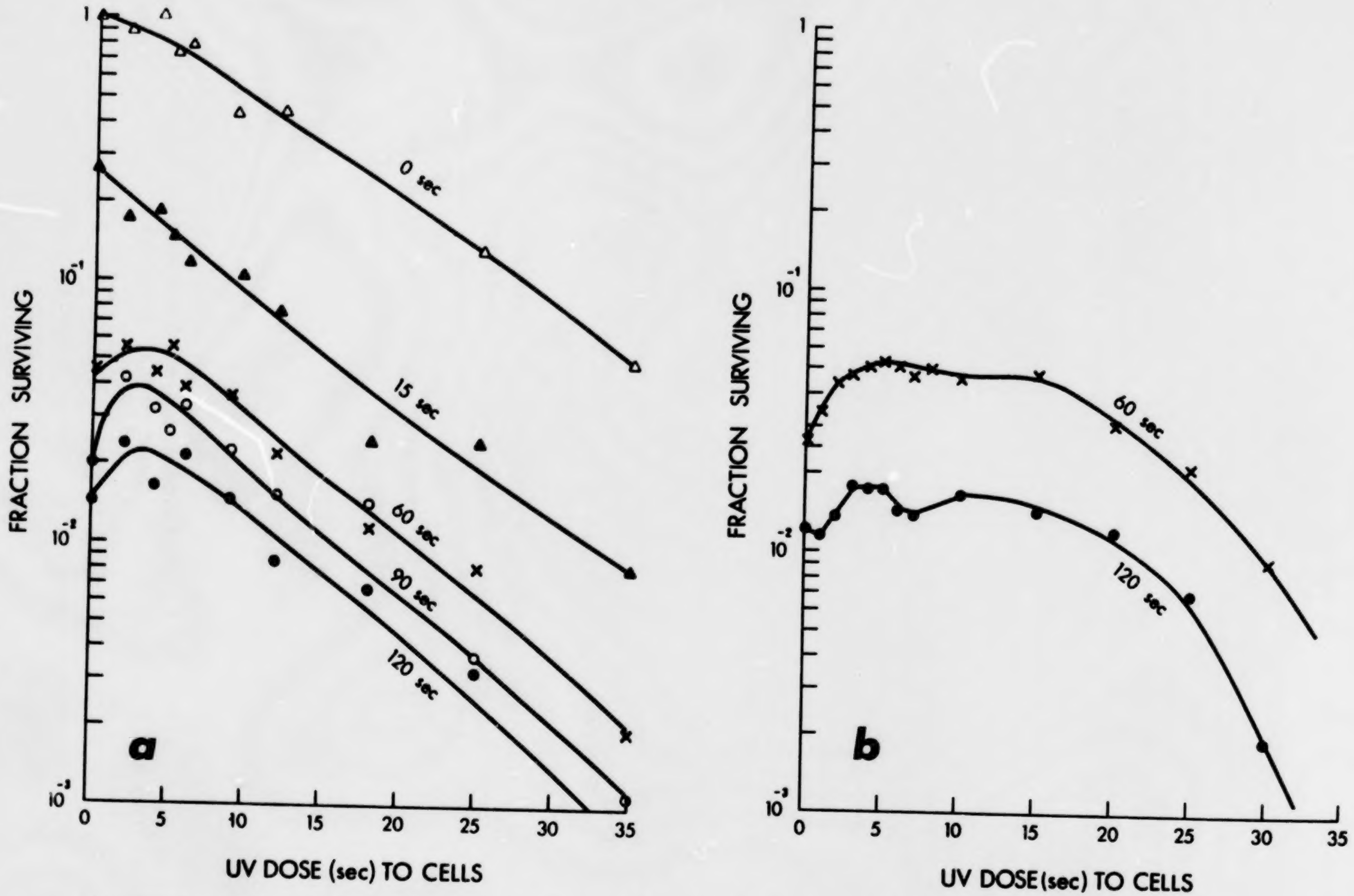

Figure 1. (a) UV-Reactivation of Herpes Simplex Virus on CV-1 Cells. Virus and cells were irradiated separately. The virus exposure (in seconds) is indicated for each curve. The incident dose rate used for irradiation of cells and of virus was 6 and 34 ergs per mm^2 per second, respectively. (b) Same experiment repeated in triplicate for virus exposures of 60 and 120 seconds.

G. A PILOT STUDY OF EFFECTS OF MICROWAVE EXPOSURE ON ONTOGENESIS

James N. Shively with the assistance of Dosimetric Studies Section, Animal Care Unit, and Pathologic Studies Section

Exposure to microwave radiation has been reported to suppress the process of maturation of lens epithelium (1), and to inhibit cellular differentiation in the developing chick embryo (2). Also, an effect of microwave exposure on differentiation of insect pupae has been reported (1). In view of these reports and the development of additional interest in the biological effects of microwave exposure, it became worthwhile to study the response in a species of immature mammal. Newborn dogs were selected for study since their retina (3) and cerebellum (4) are immature at birth and since the sensitivity of these organs of the dog to damage by exposure to ionizing radiation has been demonstrated (4,5).

Materials and Methods

When 2 or 3 days old, dogs were positioned facing a 2450 MHz microwave source and exposed for the times indicated in Table 1. The dogs were restrained to prevent undue movement. The source-to-head distance was 50 cm and the intensity of the source was 100 or 300 mW/cm^2. After exposure, the dogs were returned to their dam for rearing until tissues were collected.

TABLE 1. ANIMALS EXPOSED TO MICROWAVES

Power Intensity, mW/cm^2	Duration of Exposure, minutes	Tissue Collection, days after exposure	Number of Dogs
100	3	3	1
	3	70	3
	6	3	1
	6	70	2
	12	3	1
	12	70	2
	24	1	2
	24	70	2
300	6	70	2
	6	-[a]	2
0 (sham)	6 or 12	70	4

[a] 1 missing; 1 dead approximately 2 minutes after exposure.

At the indicated times after exposure the following tissues were collected for histopathologic study: Eye, brain, ear, teeth, endocrine organs, heart, lung, spleen, liver, kidney, gonads, stomach, duodenum, and colon. Eyes were collected from four dogs for study by electron microscopy.

Results

Only incidental morphologic changes were found. These included:

(1) Small ultimobranchial cysts were in the thyroid of two dogs (1 dog killed 70 days after 6 min. exposure to 100 mW/cm^2 and 1 dog killed 70 days after 6 min. exposure to 300 mW/cm^2).

(2) Craniopharyngeal cysts were in the anterior hypophysis in two dogs (1 dog killed 70 days after 6 min. exposure to 100 mW/cm^2 and 1 dog killed 70 days after 6 min. exposure to 300 mW/cm^2).

(3) Mild, focal myocarditis was in two dogs (1 dog killed 70 days after 6 min. exposure to 100 mW/cm^2 and 1 dog which died immediately after between 6 and 12 minutes exposure to 300 mW/cm^2. In the latter case, the age of the lesion indicated that the myocarditis existed before the initiation of microwave exposure).

(4) Rhinitis (1 dog killed 70 days after 6 min. exposure to 100 mW/cm^2. Plant material was present in the airways of this dog.)

(5) Small splenic infarcts were in two dogs (1 dog killed 70 days after 12 minutes exposure to 100 mW/cm^2 and 1 dog killed 70 days after sham irradiation).

(6) Focal, pulmonary subacute inflammation in two dogs (1 dog killed 70 days after sham irradiation and 1 dog which died immediately after exposure - see 3 above).

(7) Thoracic abscess (1 dog killed 70 days after 6 min. exposure to 100 mW/cm^2).

Subcellular changes were not detected in the retinas studied by electron microscopy.

Summary

Under the conditions used in this pilot experiment, morphologic changes attributable to microwave exposure were not found.

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H. RADIATION-ESTROGEN, POSSIBLE CO-CARCINOGENS

Frank Geiger

Both x irradiation and estrogen administration have increased mammary tumor incidence in susceptible rats. It has also been shown that whole-body exposure of x rays qualitatively induces the neoplastic response (mammary gland) and the ovary modifies this response in a quantitative fashion. The object of our experiments is to determine whether continuous exogenous estrogen increases mammary tumor incidence when rats are given whole-body x irradiation.

In a preliminary study, 120 female Fisher rats (42 ± 3 days old) were divided into eight groups so that four different levels of estrogen (Beta-estradiol) were given in combination with 2 levels of whole-body x irradiation. The levels of estrogen were 0, 0.1, 1, and 10 micrograms per day. The levels of x irradiation were 0 and 300 rads to the whole body.

The rats were fed estrogens in their diets for 10 days at which time they were irradiated and were allowed to continue their treatment diets until sacrifice 22-26 days later. Complete autopsies were done in all rats. In addition, pituitaries, ovaries, and uteri were removed, and weights were obtained in the latter two organs. There was a significant increase in ovarian weights, but no change in uterine weights. The increase in ovarian weights was an estrogen effect; however, it was the reverse of the result expected, and may have been the result of using prepuberty animals or poor absorption of the drug. Only one animal died in this initial study and it was in the control group.

I. CHARACTERIZATION OF RAT MAMMARY TUMOR CELLS IN VITRO AND IN VIVO: FURTHER OBSERVATIONS

Kiki B. Hellman

Transplantable mammary tumors in the rat have been described by Noble and Cutts (1). The most widely known is the Walker carcinosarcoma 256 which is composed of three cell types and may induce carcinoma, sarcoma, or mixed growth (2,3). More recently, the transplantability of a radiation-induced mammary carcinoma has been studied by Engle and Spencer (4). Although the tumor has retained its epithelial morphology, it was diagnosed as an undifferentiated carcinoma after the 19th transplant.

In addition to the in vivo studies, rat mammary tumor tissue has been cultivated in vitro (5,6,7). However, since more than one cell type may be present in mammary tumor tissue, it has been difficult to determine whether the cells in vitro originated from the carcinoma cells of the tumor or were malignantly transformed stroma cells of the carcinoma (7).

This report describes the in vitro growth and morphological characteristics of rat mammary tumor cells derived from a radiation-induced papillary cystadenoma, and their subsequent tumorigenicity when inoculated into the Osborne-Mendel rat, the host of origin. Similarities and differences between cells growing in the in vitro and in vivo states are presented.

A 2-month-old female Osborne-Mendel rat was irradiated with a single whole-body exposure of 450 R. Six months after irradiation, the rat developed a mammary tumor in the right axillary region. The tumor (3.0 cm in diameter) was well circumscribed, localized, and filled with 1.0-2.0 ml of serosanguineous fluid. After aseptic removal, a section of tumor was fixed for histology and the remainder used for the preparation of cell cultures.

The mammary tumor cell cultures (designated RMT) have been passed successfully through 82 transfers. Cultures are routinely maintained in MEM medium containing 2X the concentration of amino acids and vitamins supplemented with 10% newborn agamma calf serum and 100 units/ml each of penicillin and streptomycin. In order to determine the effects of different transfer methods, certain cultures were transferred by mechanical scraping (RMT-A) while trypsinization was continued with others (RMT-D). Cultures were transferred every 7 days with a medium change on the third day.

In the studies of tumorigenesis, 1 x 10^7 viable RMT cells in a volume of 1.0 ml were inoculated intramuscularly in the left thigh of 3-week-old weanling female Osborne-Mendel rats. Since whole-body x irradiation lowers the resistance of the host to tumor implants (8), both irradiated and unirradiated animals were studied. Three to four hours prior to cell inoculation, some animals were irradiated with a single whole-body exposure of 350 R.

All animals were palpated weekly for evidence of tumor. Tumors were graded from 1+ (0.5-1.0 cm in diameter) to 3+ (2.0-3.0 cm in diameter); however, some tumors greatly exceeded 3 cm. All experiments were conducted for 8-9 weeks.

RMT Cell Morphology

The initial cell cultures consisted primarily of epithelial-like cells and a small amount of connective tissue. An arrangement of cells into acinar-like structures became evident on the seventh day when a complete monolayer had formed. At the third passage, cultures were labeled arbitrarily RMT-A or RMT-D and transferred either by trypsinization or mechanical scraping in an attempt to preserve the epithelial-like cell morphology and acini. RMT-A cultures were transferred by scraping with a rubber policeman, whereas RMT-D cultures were trypsinized.

At the ninth passage, differences in morphology were observed in the RMT-D cultures. The cells, although epithelial-like, were much larger, flat, translucent, somewhat granular and had an ill-defined cell membrane (Fig. 1); acini were no longer observed in these cultures. Cell morphology stabilized at this point, remaining relatively unchanged to the present time. The RMT-A cells appear to be pure cultures of small polygonal epithelial-like cells (Fig. 2) similar to the initial culture. However, the acinar-like structures, which were observed routinely through 60 passages (Fig. 3), have been noted less frequently in more recent transfers. Connective tissue was no longer observed in either the RMT-A or RMT-D cultures after the 9th transfer. Both cell types are now in their 82nd in vitro passage.

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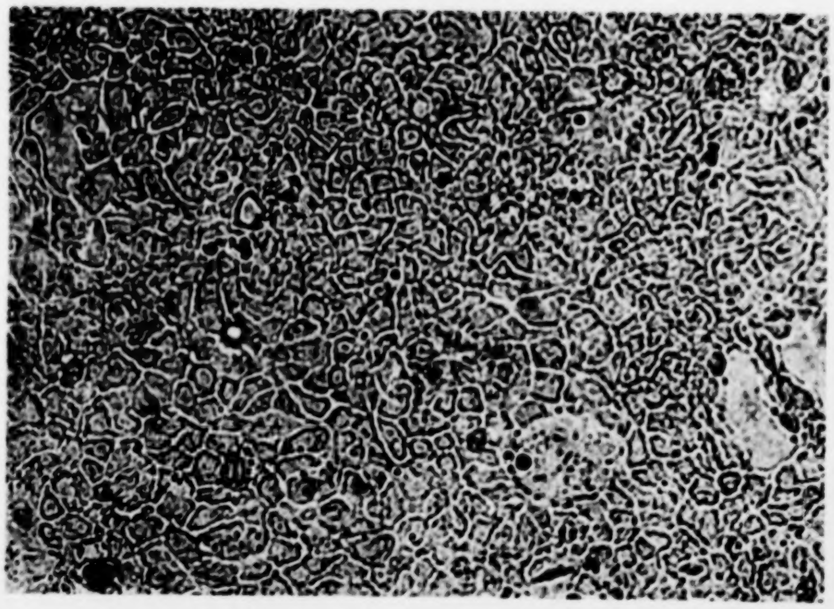

Figure 1. Light Micrograph of RMT-D Monolayer Cell Culture at the 81st In Vitro Passage. X590

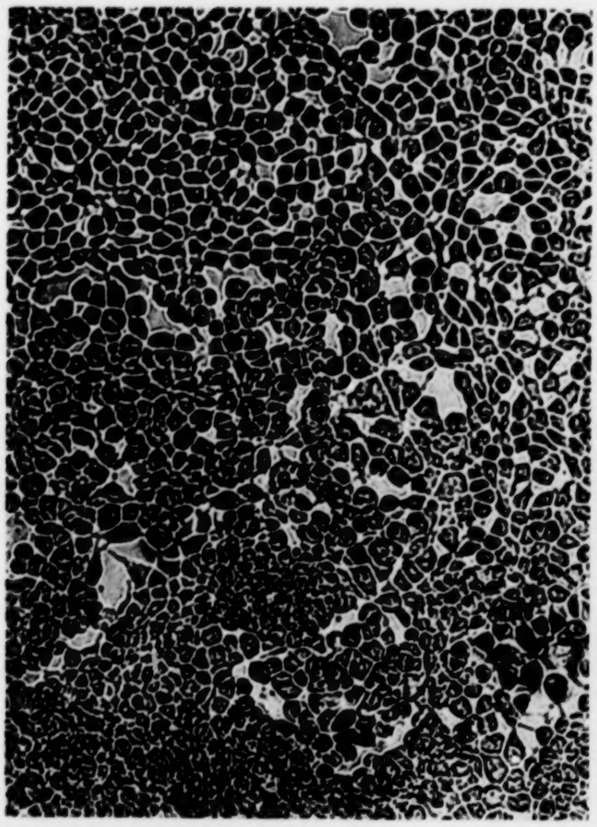

Figure 2. Light Micrograph of RMT-A Monolayer Cell Culture at the 80th In Vitro Passage. X 590

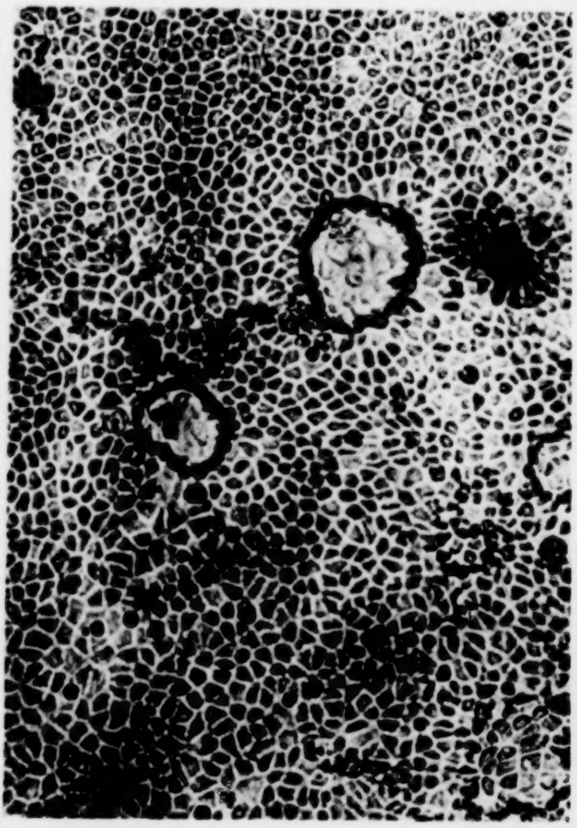

Figure 3. Light Micrograph of RMT-A Monolayer Cell Culture at the 4th In Vitro Passage. Acinar-like structures are present. X200

In an attempt to determine whether the morphological characteristics were reversible, RMT-A cultures were transferred with trypsin; this resulted, at the tenth transfer, in a morphological appearance similar to the RMT-D cultures. Repeated attempts to transfer RMT-D cultures by scraping with a rubber policeman have been unsuccessful, the cells failing to attach and proliferate.

Growth Curve Studies

Since differences in morphology and pattern of growth became evident as a result of differences in handling of cultures, it was of interest to determine if differences in rate and extent of growth also existed. As is noted in the representative growth curve (Fig. 4), the generation time of RMT-D cultures is 24 hours compared to 20 hours for RMT-A. Although the difference in generation time is slight, the absence of an appreciable lag phase and a greater extent of growth is observed in RMT-A cultures. Harvests of RMT-A cultures at 7-8 days yield anywhere from 1.4 to 1.8 x 10^6 cells/ml compared to 9 x 10^5 to 1.0 x 10^6 cells/ml for RMT-D cultures.

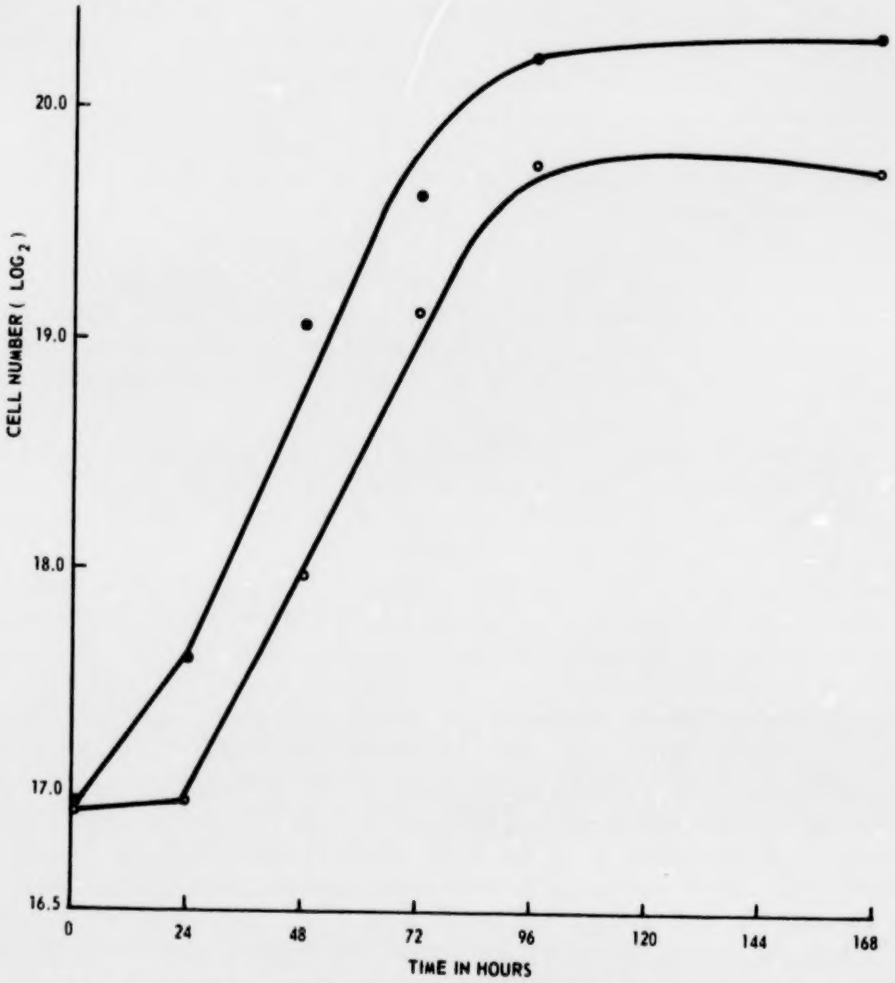

Figure 4. Growth Curve of RMT-A and RMT-D Monolayer Cultures. Each point represents the average of cell counts from 3 replicate cultures. RMT-A cells (●), RMT-D cells (○).

Development of RMT Tumors

The primary tumor was classified, histologically, as a papillary cystadenoma. Papillar projections into the cystic lumen were observed and, as is noted in Fig. 5, epithelial-like cells lined tubules and ducts against a stroma of connective tissue.

Both RMT-A and RMT-D cell cultures passed through 7, 18, 36, 53, and 76 in vitro passages were inoculated into Osborne-Mendel rats, in attempts to correlate growth rates and morphological differences with differences in tumorigenicity. Early (7, 18 transfers) as well as later cultures (36, 53, 76 transfers) of both cell types produce palpable tumors of a 1+ size 7 days after inoculation in 100% of unirradiated animals. Tumors continued to grow to a 3+ stage and then regressed at 14-18 days in animals inoculated with RMT-D cells. RMT-A cells gave rise to tumors which grew at a greater rate and persisted for 2-3 weeks longer; in some instances, tumors were 5.0 cm in diameter before regressing. As is noted in Table 1, not only do RMT-A cells induce tumors which persist for a greater length of time, but they also produce tumors with a smaller cell inoculum (1×10^5 RMT-A cells induced tumors in 5/15 animals, whereas 1×10^6 RMT-D cells are required to initiate tumors). In addition, the greater growth rate accounted for 2/15 deaths in animals receiving 1×10^7 RMT-A cells.

Depressing the host's immune response by means of x irradiation and subsequent inoculation with 1×10^7 RMT cells permitted a greater growth and persistence of tumor. RMT-D tumors grew to 4.0-5.0 cm in diameter before regressing at 21-30 days. Regression was noted less frequently in RMT-A tumors, the greater tumor growth (6.0-7.0 cm) resulting in animal death (tumors persisted for 2-3 months in some cases before causing death). The latent period of 7 days remained unchanged.

Tumors induced by RMT-A and RMT-D cells at different in vitro passages were morphologically similar to the original, primary tumor. They were well circumscribed and limited to the area of cell inoculation. Metastasis to the lung occurred in two animals (Fig. 6). Otherwise, all other organs appeared normal with the exception of spleen enlargement in animals bearing larger tumors. All induced tumors were cystic, being filled with a serosanguineous fluid.

Histologically, tumors induced by both RMT cell types were diagnosed as papillary cystadenomas. As can be noted in Fig. 7, tumors induced by RMT cell cultures of earlier passages were identical to the primary tumor. Although both RMT-A and RMT-D cell cultures of later passages produced tumors which were not as well differentiated as the primary tumor, epithelial-like cells predominated which tended to form a pattern suggestive of ducts and acini (Fig. 8 and 9). Connective tissue was evident and seemed to be more pronounced in tumors induced by RMT-D cells.

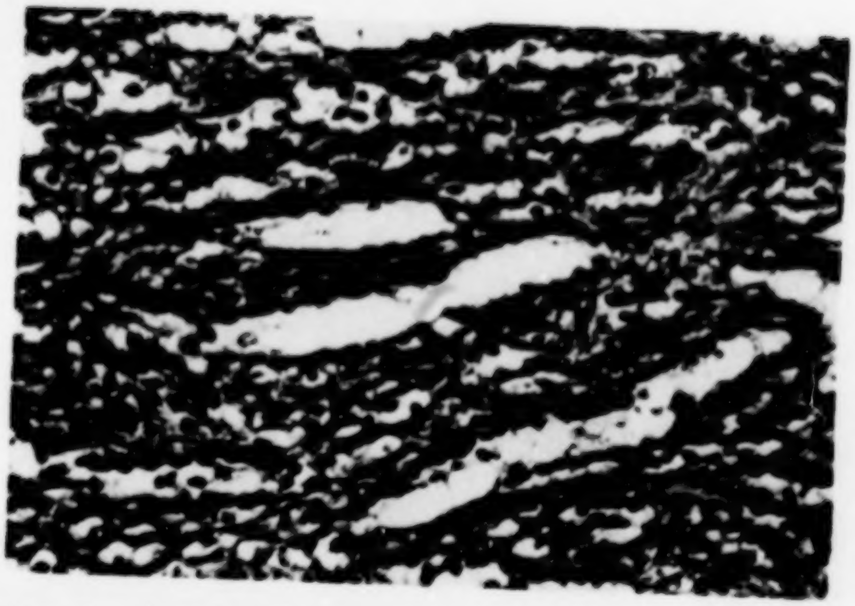

Figure 5. Section Through the Primary Tumor Showing Irregularly Shaped Acini Lined by a Layer of Epithelial Cells. Hematoxylin and eosin. X300

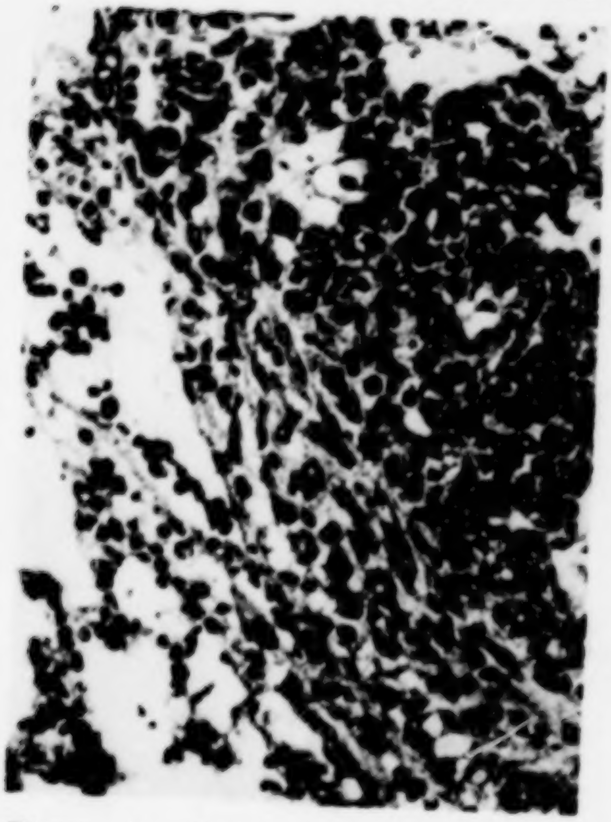

Figure 6. Section Through Lung of Tumor-Bearing Animal Showing Metastasis. Note islands of epithelial-like carcinoma cells in lung tissue. Hematoxylin and eosin. X300

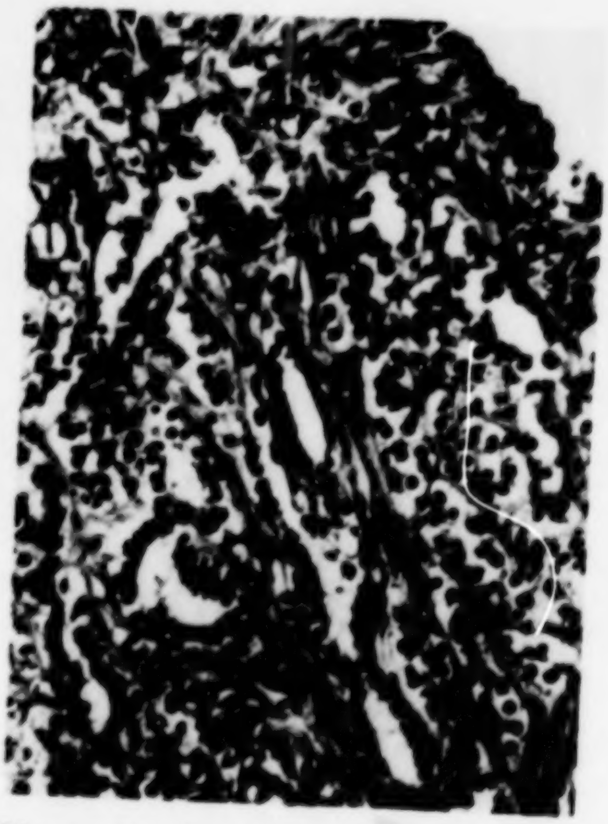

Figure 7. Section Through Mammary Tumor Induced by RMT-D Cells (7 in vitro passages; 1st return of cells to the animal). Note similarity to primary tumor. Hematoxylin and eosin. X300

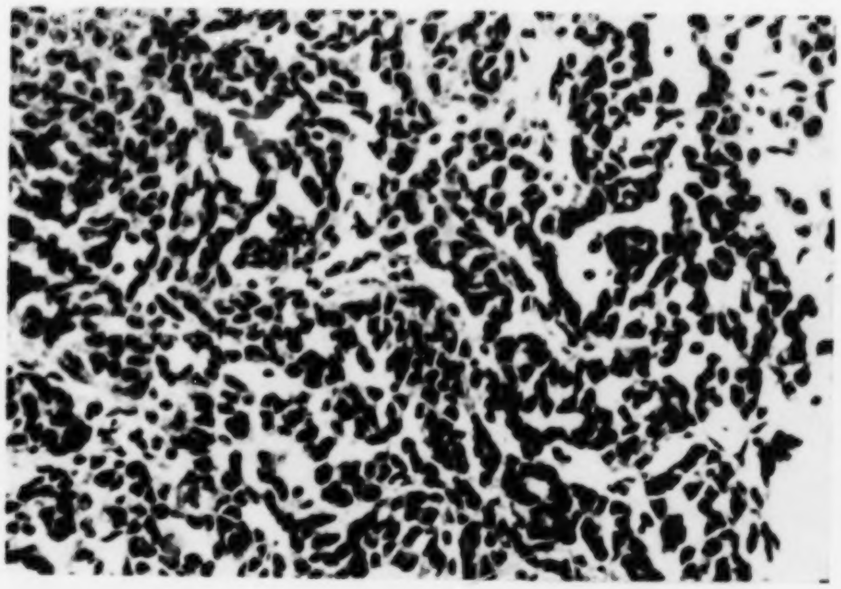

Figure 8. Representative Histological Section of Mammary Tumor Induced by RMT-A Cells. Hematoxylin and eosin. X300

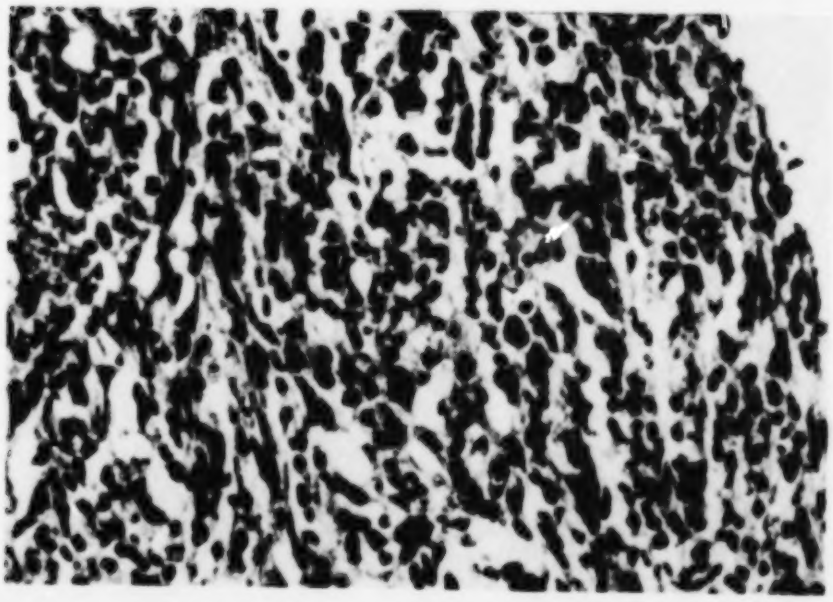

Figure 9. Representative Histological Section of Mammary Tumor Induced by RMT-D Cells. Hematoxylin and eosin. X300

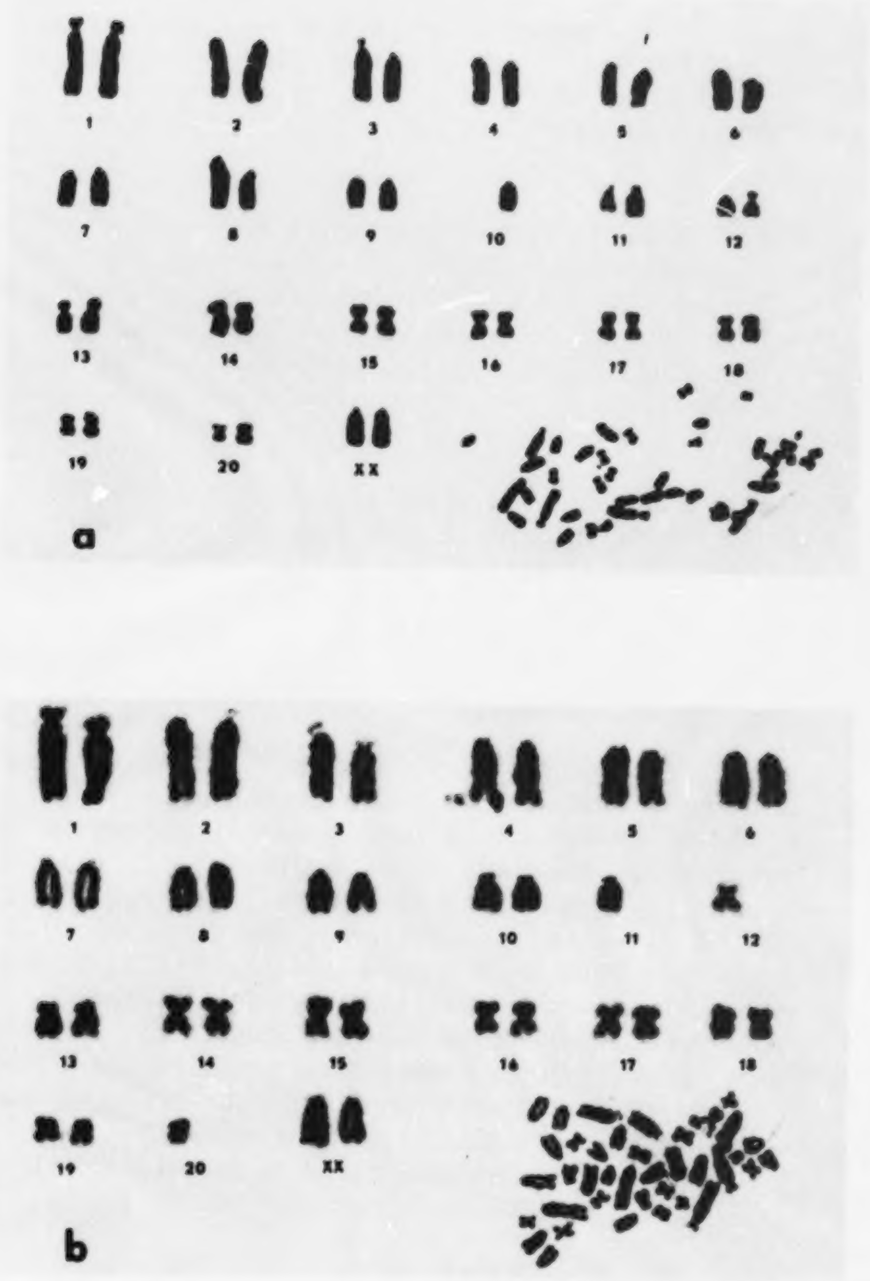

Figure 10. Karyotypes of RMT Cells Cultured In Vitro. (a) Karyotype of RMT-A at 20th passage; (b) karyotype of RMT-D at 21st passage. Note Structural alteration (elongation) of No. 3 chromosome in both a and b.

Chromosomal Analysis

In collaboration with the Genetic Studies Section, chromosomes of RMT-A (20th transfer) and RMT-D (21st transfer) cells were analyzed in order to determine if cell morphology or tumorigenic properties could be correlated with differences in either the number or structure of the chromosomes.

Sixty-six chromosome spreads were observed in RMT-A, and fifty-five in RMT-D. Karyotypes were prepared according to the system of Hsu and Benirschke (9). The normal diploid number of chromosomes (2n=42) was observed in 76 percent (50/66) of RMT-A cells and 60 percent (30/52) of RMT-D cells. Karyotypes of hypodiploid cells revealed no consistently absent chromosome in either cell line. Tetraploidy was present in approximately 3 percent of the cells of both types.

Structural differences between the homologues of the subtelocentric No. 3 chromosome pair as noted in Figures 10a and 10b were identified in 50 percent of the karyotypes of both RMT-A and RMT-D cells and suggested in the remaining cells, although chromosome position prevented positive identification. The long arms of one chromosome of the homologous pair were shorter by a factor approximately equal to the length of the short arms of these chromosomes.

Reports on the association of virus particles with rat mammary tumors have been varied. Pollard's (10) report on electron microscopic examination of x-irradiated and DMBA-induced carcinomas in germ-free rats revealed no C-type particles. However, since Engle, et al. (11) recently reported on the presence of C-type virus particles in a transplantable and x-irradiation-induced rat mammary carcinoma, and Chopra's (12) study revealed C-type particles in both spontaneous and chemically induced rat mammary tumors, it was of interest to investigate the presence of viral particles in the system reported in this study. Normal rat mammary tissue, tissue from the primary tumor, tissue from mammary tumors induced by both RMT-A and RMT-D cell types, and extracts from RMT cell cultures have yielded no C-type particles when examined by electron microscopy. In addition, cell-free extracts prepared according to Scholler and Bergs (13) and subsequently inoculated into newborn rats failed to induce tumor development.

Summary

Permanent cell cultures have been established from a radiation-induced papillary cystadenoma obtained from a female Osborne-Mendel rat. Two morphologically distinct and stable cell types were observed (RMT-A and RMT-D). Results suggest that differences in morphology and growth rate between the cell types were due to different methods of handling the cultures in vitro. Both RMT-A and RMT-D cell cultures of early (7, 18) as well as later (36, 53, 76) in vitro passages induced tumors in 100 percent of irradiated and unirradiated female Osborne-Mendel rats. Induced tumors were morphologically and histologically similar to the primary tumor and have been diagnosed as papillary cystadenomas. Differences observed in growth of tumors induced by the different cell types can be correlated with differences observed in the growth rate of cell cultures in vitro. Results suggest that the cells in vitro originated from the carcinoma cells of the primary tumor.

Utilizing results obtained from this study dealing with the minimal number of cells required to induce tumors and the differences in this relative to the cell type, experiments are now in progress to determine the effect of x irradiation both on the appearance and regression of the tumors. Since x irradiation depresses the hosts' immune mechanism, these studies will be an indirect measure of the time course of immune suppression.

TABLE 1. INCIDENCE AND REGRESSION OF MAMMARY TUMORS IN RATS[a] INOCULATED WITH RMT CELLS

Cell Inoculum	Tumor incidence[b]		Tumor Regression					
			RMT-A			RMT-D		
			Days postinoculation			Days postinoculation		
	RMT-A	RMT-D	14	18	28	14	18	28
$1x10^4$	0/15	0/15	-	-	-	-	-	-
$1x10^5$	5/15	0/15	5/15	-	-	-	-	-
$1x10^6$	15/15	4/15	-	15/15	-	4/15	-	-
$1x10^7$	15/15	15/15	-	5/15	13/15[c]	12/15	15/15	-

a. Unirradiated 3-week-old female Osborne-Mendel rats were inoculated i.m. with either RMT-A or RMT-D cells.

b. Tumor incidence 7 days postinoculation.

c. 2/15 animals dead from tumor growth.

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J. CATARACTOGENESIS OF 30 KVP AND 250 KVP X RAYS

James N. Shively, James W. Rolofson, Clayton F. Cisar and Seth Koch[1]

Introduction

Induction of lenticular opacities has been recognized for many decades as a hazard of exposure to undue amounts of ionizing radiation. A vast amount of literature about ionizing radiation-induced cataractogenesis exists; however, almost all of these studies have used laboratory rodents exposed to high-energy neutrons, x, or gamma rays.

With the advent of interest in the potential hazard from electronic devices emitting less energetic x rays, an attempt to evaluate induction of cataractogenesis by these sources became appropriate. Swine were selected as experimental subjects in an attempt to more nearly simulate the human eye. Since the relative effects of 30 and 250 kVp x-ray exposures were desired at an early date, the study was designed for completion in about 18 months.

Materials and Methods

Sixty weaned, mixed breed 10- to 12-week-old swine were purchased and were quarantined for 3 weeks before their eyes were irradiated. The swine were kept in a barn having concrete floors,[2] fed a commercial hog ration, and given water *ad libitum*.

Surgical anesthesia was induced by the intravenous injection of pentobarbital sodium to maintain immobility of the swine during irradiation of the ocular area. The swine were placed in lateral recumbency for irradiation and the eyelids were closed with tape. The beam was collimated and a lead shield was positioned to prevent exposure of tissue other than the globe and periorbital structures. Each eye of the swine was exposed to an absorbed dose at the midpoint of the lens of 100, 600, or 1,000 rads of 30 kVp or 250 kVp x rays.

The 30 kVp exposures were done with a low energy (0-75 kVp) x-ray machine operating at 30 kV constant potential, 90 mA, 25 cm T.S.D. with 3.09 mm aluminum filtration added to a collimated beam. The absorbed dose rate was approximately 38 rads/minute. For higher energy x irradiation, a 250 kV constant potential x-ray machine was used operating at maximum energy, 7.0 mA, 20 cm T.S.D. with a thoreaus filter and 0.42 mm lead added to the collimated beam. The dose rate at the midpoint of the eye lens was 56 rads/minute.

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[1] Veterinary Ophthalmologist, Eye Research Foundation, Bethesda, Md. and the Animal Hospital of Penn Daw, Alexandria, Va.

[2] The swine were kept at the National Institutes of Health Animal Center under contract with the Laboratory Aids Branch, National Institutes of Health.

Eyes were collected from each of three swine 3 or 10 days after exposure to 100 rads or 600 rads of 30 kVp or 250 kVp x radiation. Eyes were collected from each of six swine 1 year after exposure to 100 rads or 600 rads of 30 kVp or 250 kVp x rays and from each of two swine 1 year after exposure to 1,000 rads 30 kVp or 250 kVp x rays. The swine were anesthetized with pentobarbital sodium and the eyes were quickly extirpated. The swine were killed with the drug immediately after collection of the eyes. For electron microscopy of tissues from some of the swine, one eye was hemisected transversely just rostral to the equator and the rostral calotte was immediately placed in 6.25 percent glutaraldehyde in phosphate buffer (pH 7.3) with 2 percent acrolein added. Fixation was continued for 90 minutes after which the calotte was transferred to phosphate buffer, pH 7.3. Blocks about 1 x 1 x 3 mm were cut from the anterior lens, lens bow, posterior lens, and the central cornea and post-fixed in 2 percent OsO_4 in veronal buffer. The blocks were dehydrated in a series of ascending concentrations of alcohol and embedded in epon. Silver to grey (color) sections were cut on an ultramicrotome, stained with orange acetate and lead citrate (a few sections were stained with 1 percent phosphotungstic acid) and examined in an electron microscope operating at 80 kV potential.

For light microscopy one eye from each swine was fixed for 24 hours in the solution used for fixation for electron microscopy or for 72 hours in 10 percent formalin. The globe was hemisected dorso-ventrally and one calotte was dehydrated and embedded in paraffin or celloidin. Sections were cut and stained (H & E) for examination with the light microscope.

Periorbital tissue and ocular adnexa were collected for light microscopy and a complete necropsy was done on each swine killed.

For about 3 months after irradiation the eyes were examined with an ophthalmoscope. Then the collaboration of a veterinary ophthalmologist (SK) became available and periodic examinations by biomicroscopy and indirect ophthalmoscopy were done.

Results

After exposure to 100 rads, 250 kVp x rays, two of the six swine developed mild transient conjunctivitis. A clinical response was not detected in the swine exposed to 100 rads, 30 kVp. There was periorbital erythema and mild edema within a day after exposure to 600 or 1,000 rads. Blepharitis, palpebral edema and a mild purulent conjunctivitis developed a few days after the exposure to 1,000 rads. The leukoma occurring in the swine exposed to 1,000 rads was transient and had cleared by about 14 days after irradiation.

Mild chronic conjunctivitis developed in swine exposed to 1,000 rads and in some of the swine exposed to 600 rads. Chronic conjunctivitis occurred more frequently in swine exposed to 600 rads, 30 kVp, x rays than in those exposed to 600 rads, 250 kVp x rays; however, chronic conjunctivitis did not occur after 100 rads, 30 kVp x rays, but was seen in two swine exposed to 100 rads, 250 kVp x rays.

Transient depigmentation of hair in the periorbital area was dose-related and occurred after 600 rads or 1,000 rads. This depigmentation did not appear to be x-ray energy dependent.

Enophthalmos became apparent in both of the swine exposed to 1,000 rads, 250 kVp x rays, in one of two swine exposed to 1,000 rads, 30 kVp x rays, and in one of six swine exposed to 600 rads 250 kVp x rays. This change was not detected in any of the other swine.

Biomicroscopy and indirect ophthalmoscopy revealed equatorial vacuoles in the lens of one of two swine 3 months after exposure to 1,000 rads, 250 kVp x rays. By 4 months after exposure, a similar lesion had developed in one of two swine exposed to 1,000 rads 30 kVp x rays. Illness of the veterinary ophthalmologist then precluded definitive examination of the swine until 10 months after irradiation when all of the swine exposed to 1,000 rads had detectable posterior lenticular opacities. While occasionally a small nuclear or anterior suture opacity was seen, the only other radiation-type opacity was detected in one swine 4 months after exposure to 600 rads, 250 kVp x rays.

As expected, when swine are housed on concrete floors, some of the swine kept for the longer term study became lame. Morphologic changes in the joints were less severe than expected from the degree of lameness.

Histopathologic examination of tissues from the group of swine killed 3 or 10 days after irradiation revealed that all were infested with Stephanuris dentatus. However, lesions produced by the parasites were not found in the orbital or ocular tissues of any of the swine. A few of the swine had metastrongylus in the respiratory tissue.

Histopathologic examination of eyes collected 3 days after 100 rads of 30 kVp or 250 kVp revealed only the rare occurrence of a necrotic cell in the lens epithelium near the bow. Necrotic nuclei occurred slightly more frequently after irradiation with 600 rads of either energy, but the occurrence was infrequent. Differences in frequency or severity of the change was not detected between 30 kVp or 250 kVp x rays.

Significant morphologic changes were not found 10 days after 100 rads of either 30 kVp or 250 kVp x rays. A few necrotic lens epithelial cells were noted in eyes collected 10 days after exposure to 600 rads of either energy. There was mild corneal edema and occasionally a necrotic cell in the corneal epithelium. Some swine had mild acute to subacute conjunctivitis.

There was a tendency for the globes to be smaller in swine irradiated with 600 rads and 1,000 rads of either energy. The numbers of eyes available was too small for detailed statistical analysis, but the eyes exposed to 1,000 rads were at least 10 percent smaller in all dimensions than were the eyes exposed to 100 rads. The effect was not energy dependent.

Lesions were not found in eyes or adnexa 1 year after exposure to 100 rads of 30 kVp or 250 kVp x rays. One year after exposure to 600 rads of either energy there were a few nucleated lens fibers between the lens bow and the posterior pole. Very small discrete lesions were at the posterior pole of the lens in two swine; of these, one was exposed to 30 kVp x rays and the other to 250 kVp x rays. A few of the swine exposed to 600 rads had mild chronic inflammation of the conjunctiva of the palpebra and the nictitating membrane. These changes were not energy dependent.

Foci of nucleated lens fibers, degenerate fibers and balloon cells were at or near the posterior pole of the lens from swine exposed to 1,000 rads of either energy x rays. There were nucleated lens fibers between the lens bow and the posterior pole. Focal, active chronic blepharitis and conjunctivitis occurred in these swine and there was focal hyperplasia of mucous secreting cells on the nictitating membrane. Moderate hyperkeratosis was seen. The lesions were not significantly different in the two energy groups.

Study of subcellular structure of the lens from a few of the eyes confirmed the light microscopy data, but did not provide additional information.

Discussion

Results indicate that the eyes of swine are similar to other large mammals in sensitivity to radiation-induced lenticular opacities. Both the dose relationships, the latent period, and development of the lesions resembled those produced in other large mammalian species.

The study was designed to investigate relative cataractogenesis of 30 kVp and 250 kVp x rays. The absence of significant morphologic changes after 100 rads of either energy x rays indicates that the lens is not uniquely sensitive to damage by low energy x rays.

The mild morphologic changes after 600 rads provided an excellent comparison of minimal lenticular response to the two energies. Similarity of the lesions occurring in lens after exposure to either of the x-ray energies indicates that at least under the conditions of this study the mammalian lens is not more sensitive to low energy x rays.

Similarity of the time of onset and the severity of lesions occurring after exposure to 1,000 rads of either 30 kVp or 250 kVp x rays provided additional evidence that the mammalian lens is not unduly sensitive to low energy x rays.

Summary

Morphologic study of eyes of swine 3 days, 10 days, or 1 year after exposure to 100, 600, or 1,000 rads 30 kVp or 250 kVp x rays did not indicate energy dependency of the lesions seen.

NEUROPHYSIOLOGIC STUDIES SECTION
Dr. Moris L. Shore, Acting Chief

The Neurophysiologic Studies Section has continued to develop methods and procedures to investigate the possible neurological effects of radiation in the microwave frequency range. This effort has resulted in the development of a simple and practical method for determining the power absorbed in a biological system. In addition to this effort, a program was begun in mid-year to determine the effects of microwave radiation on behavior. This program is currently being staffed and equipped.

A. MICROWAVE DOSIMETRY USING A RESONANT CAVITY
William Edwards

Background

It is intuitively plausible that in order for microwaves to have an effect on the central nervous system, microwave power must be deposited in CNS tissue. However, because of the present state of the art, power measurements cannot be obtained from the CNS of living organisms. Rather, power densities per unit area are measured and absorbed power is calculated.

In order to avoid the difficulties and inherent inaccuracies of such calculations, a technique has been developed which simplifies the determination of power absorption by the head of a conscious rhesus monkey. The system employs a resonant cavity which is essentially a radio frequency (RF) closed system in which power delivered to the system (incident power) is either absorbed or returned to the source (reflected power). The difference between the incident and reflected power is related to the Q parameters of the cavity. Changes in the Q parameters of the cavity are related to the shift in the resonant frequency brought about by the cavity load created when the head of a monkey is inserted into the cavity.

Q and the Load

The quality of the unloaded cavity (Q_o) can be related to at least two parameters of direct interest: (1) The maximum ability of the cavity to store energy compared to its losses;

$$Q_o = \frac{2\pi \text{ energy stored}}{\text{energy loss per cycle}}$$

(2) the bandwidth of the cavity resonator

$$Q_o = \frac{f_o}{f_2 - f_1}$$

where f_2 and f_1 are the upper and lower half-power frequencies, respectively, and f_o is the resonant frequency.

The power losses in the total cavity system before the monkey's head is placed inside are compared with the losses after the head is inserted. It is not necessary to distinguish the particular property of the cavity from that of the input coupling network; therefore, the term, Q open circuit, refers to the Q value of the cavity measured with the input coupling positioned and adjusted. This value of Q is called (Q_c).

When the animal head is placed in the cavity, a new value of Q is determined, called Q loaded (Q_L). The losses represented by the animal head can likewise be expressed in terms of Q, called Q external (Q_m).

The following relation exists between the three different values of Q:

$$\frac{1}{Q_L} = \frac{1}{Q_c} + \frac{1}{Q_m}$$

This relationship simply states that the total loss is equal to the internal cavity losses plus the head or external circuit losses. The total energy losses represent the impedance of the load terminating the transmission line. Impedance here refers to the existence of standing waves as a consequence of partial absorption of the energy in the incident wave at the load. The generator or source end is always uniformly matched to the line. The use of the word load refers to the impedance at a plane in the line considered to be the feed point of the coupling network. This position establishes a reference plane for the further evaluation of absorbed power by the animal's head. The impedance seen here appears as a series-resonant impedance connected in series with the reactance of the coupling network (see Figures 2-4). The coupling network is designed to show negligible losses even though it is not considered as a separate element in the derivation of the dose. When the cavity is operating at the excitation frequency called the mid-point shift frequency, this impedance becomes completely resistive and represents (1) the original losses of the cavity plus the additional losses due to the animal head and (2) the complete resistive component of the complex load impedance terminating the line. The magnitude of this load impedance is determined by two basic parameters, the particular reference plane chosen and the strength of the coupling between the cavity and the transmission line. By varying the strength of the coupling, a virtually matched load can be presented to the line. A voltage standing wave ratio (VSWR) of less than 1.3 has been experimentally measured.

Load Determination

By measuring changes in the parameters of the cavity, transmission lines, and frequency shift, the equivalent circuit shown in Figure 1 was devised leading to a mathematical insight of the cavity functions and a clearer description of the power distribution.

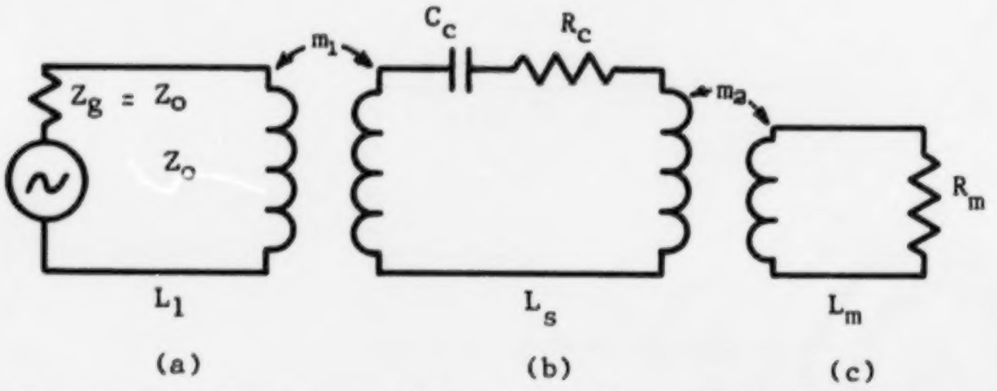

Figure 1. Equivalence Circuit of the Irradiation System.

In Figure 1, section (a) represents the source or input circuit, section (b) the cavity, and (c) a circuit which causes the same effects as those observed by the insertion of the animal head. By transferring the complex impedance of the coupling network to the cavity and adding their respective components, the equivalence circuit shown in Figure 2 can be drawn.

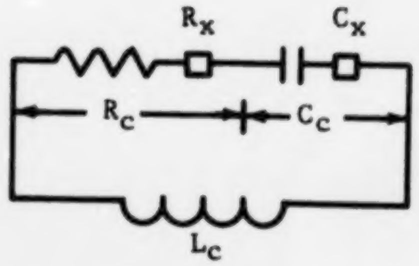

Figure 2. The Effect of the Coupling Transfer to the Cavity Parameters.

In the present case, these components did not change the cavity parameters significantly and the impedance equation for the condition in which the head is placed in the cavity is essentially the same. If Z_c is the series impedance of the equivalence circuit and j is $\sqrt{-1}$, the following equation can be written:

$$Z_c = R_c \left[1 + jQ_c \left(\frac{\omega^2 - \omega_o^2}{\omega^2} \right) \right] \tag{1}$$

Therefore the cavity can be made to appear resistive as the excitation frequency (ω) approaches the resonant frequency (ω_o). The magnitude of this loss can be defined simply as R_c. Placing the head in position is in effect transferring the impedance of circuit (Figure 1,c) to the circuit of Figure 2. Redrawing and writing the equation for the transferred impedance of circuit (c) produces Figure 3 and Equation 2, where $Z_{m'} = R_{m'} - jR_{m'}Q_m$, the impedance referred to the cavity by the animal head.

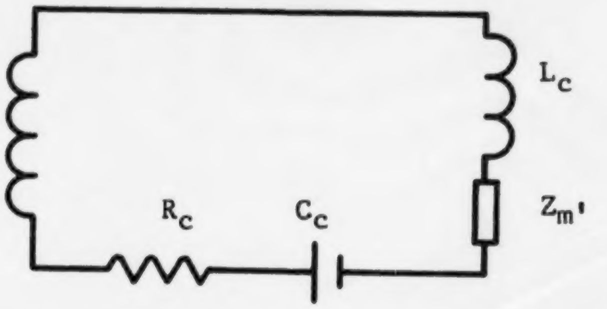

Figure 3. The Equivalence Circuit of the Head Referred to the Cavity.

$$Z_c = R_{m'} + R_c + j\omega L_c - j\frac{1}{\omega C_c} - jR_{m'}Q_m \tag{2}$$

The change in the value of the capacitive reactance now seen by the cavity in Equation (2) results in a shift in the resonant frequency of the loaded cavity.

$$Z_c = R_{m'} + R_c \left[1 + jQ_c \left(\frac{\omega_o^2 - \omega_o^2}{\omega^2} - \frac{\omega_{oL} - \omega_o}{\omega^2} \right) \right] \tag{3}$$

Figure 4 shows a plot of the frequency tuning parameter as a function of the frequency of excitation for the conditions where (ω_o) is equal to 380 MHz and (ω_{oL}) the shifted resonant frequency is equal to 383 MHz.

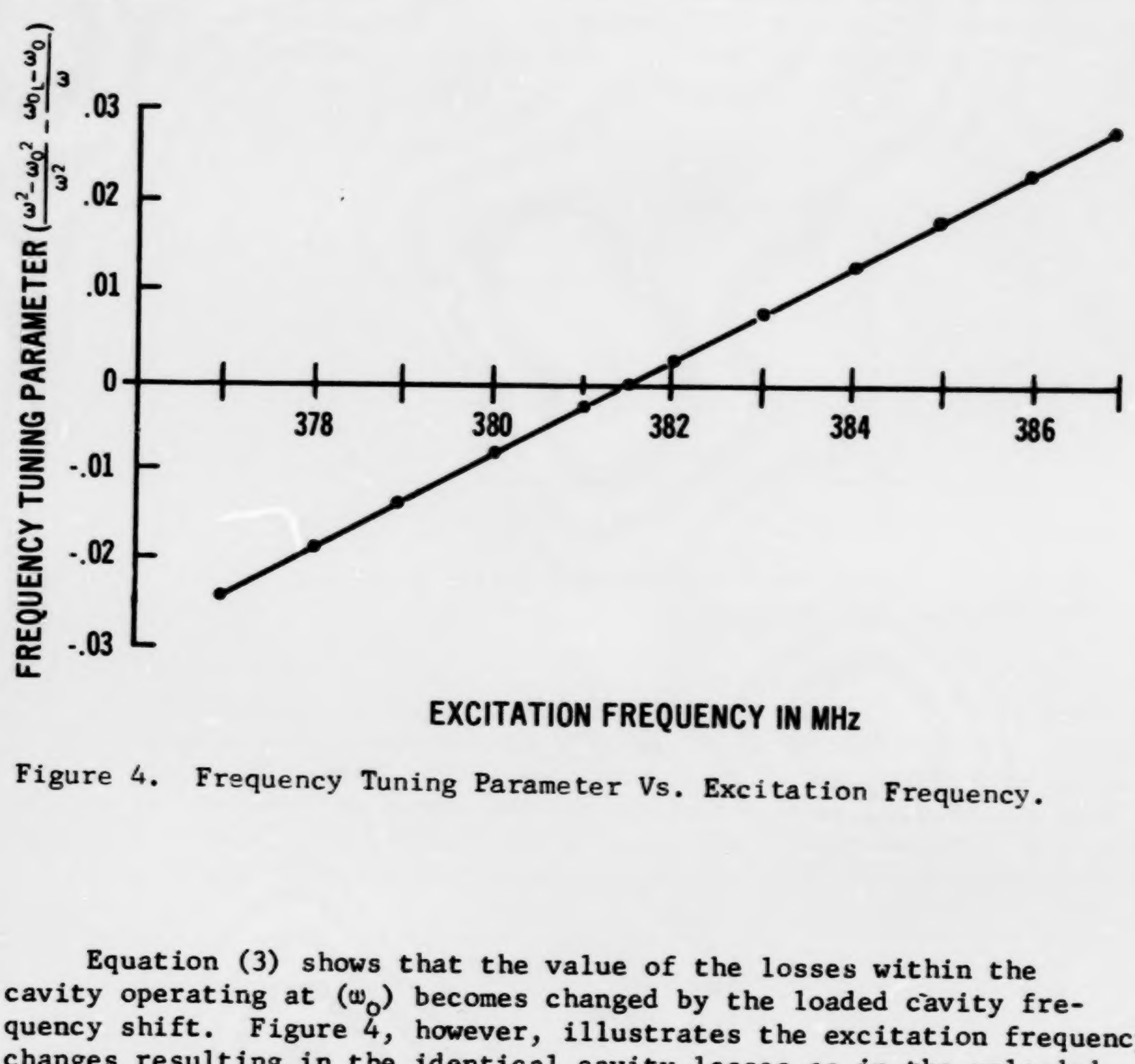

Figure 4. Frequency Tuning Parameter Vs. Excitation Frequency.

Equation (3) shows that the value of the losses within the cavity operating at (ω_o) becomes changed by the loaded cavity frequency shift. Figure 4, however, illustrates the excitation frequency changes resulting in the identical cavity losses as in the unloaded (R_c) case plus the losses of the animal head ($R_{m'}$). (The total reactive components approach zero.) The new excitation frequency is midway between the loaded and unloaded resonant frequencies. If this frequency is used, the series impedance of the cavity can again be written simply as:

$$Z_c = R_c + R_{m'} \tag{4}$$

Power Distribution

The impedance of the loaded cavity as expressed in Equation (4) is transferred to the input circuit (Figure 1,a), and the degree of coupling is adjusted to yield a virtually matched line (VSWR is 1.5 or less). This portion of the circuit in Figure 1 is redrawn to yield Figure 5.

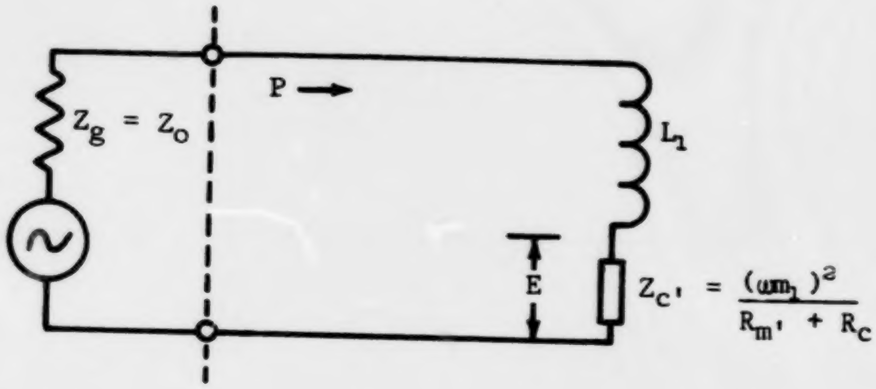

Figure 5. (Z_c') the Total System Losses Referred to the Assumed Transmission Line Feed Point.

The transmission line with a VSWR of 1.5 or less is considered a non-resonant line and the ratio of voltage to current is everywhere nearly equal to the characteristic impedance of the line. Therefore the voltage at the load is proportional to the power as measured (see Figure 6). Since this power is absorbed by the resistance (Z_c') the components are available by considering the conductance (Figure 7).

$$\frac{1}{Z_c'} = \frac{R_m'}{(\omega m_1)^2} + \frac{R_c}{(\omega m_1)^2} \tag{5}$$

Since $R_c/(\omega m)^2$ is the same as the unloaded cavity operating at (ω_o), it can be obtained by measurements when the animal's head is removed from the cavity. Therefore the power absorbed by the head is

$$P_{mH} = P_L - \frac{E^2 R_c}{(\omega m)^2}$$

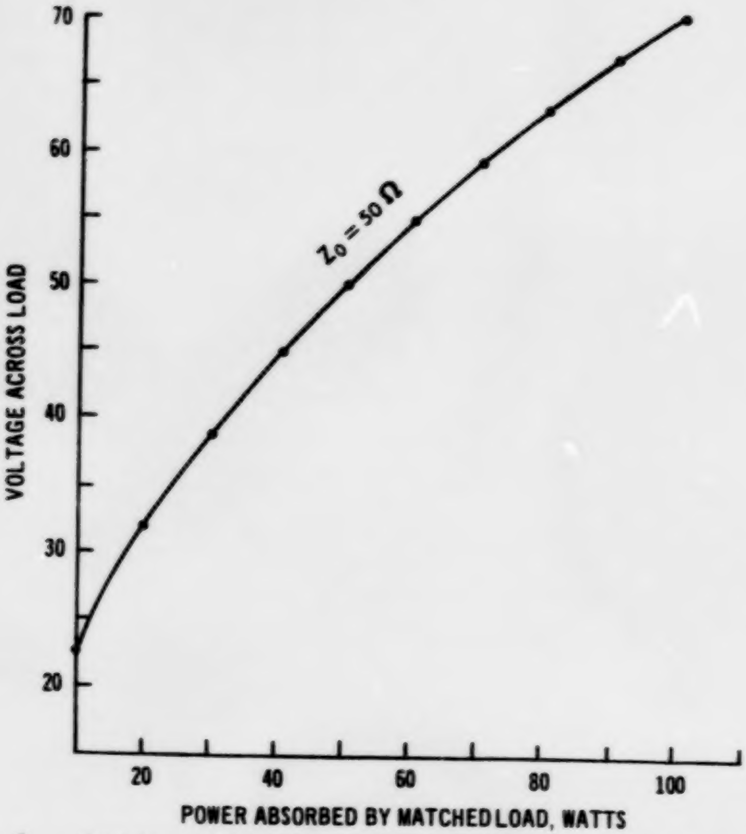

Figure 6. Voltage Vs. Power in a Matched Transmission Line.

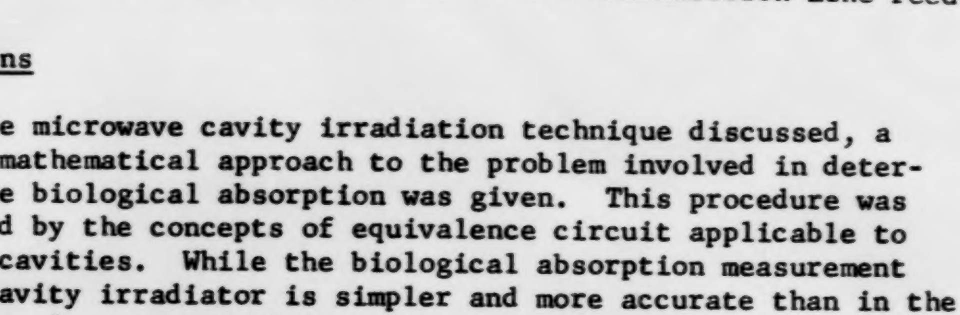

Figure 7. Load Conductance Referred to the Transmission Line Feed Point.

Conclusions

In the microwave cavity irradiation technique discussed, a detailed mathematical approach to the problem involved in determining the biological absorption was given. This procedure was simplified by the concepts of equivalence circuit applicable to resonant cavities. While the biological absorption measurement for the cavity irradiator is simpler and more accurate than in the free-space plane-wave technique, there are other measurements of interest that are more complicated and technically more difficult. However, the microwave cavity irradiator will play an important role in the understanding of microwave frequency energy effects on biological systems.

GENETIC STUDIES SECTION
Dr. William M. Leach, Chief

The research of the Genetic Studies Section during 1971 sought to increase observations of chromosomal injury in circulating lymphocytes of ionizing radiation-exposed humans, to determine genetic and cytogenetic responses of metazoans to microwave radiation, to develop biochemical screening techniques for mutations in radiation-treated cell populations, to investigate possible relationships between chromosomal changes and cellular carcinogenesis, and to provide direct cytogenetic support for other research groups in the division. The following summaries of individual studies provide a measure of the extent of utility and success of genetic and cytogenetic research in relation to the bio-effects program in support of a national radiological health program.

A. HUMAN STUDIES

Microwave Irradiation of Peripheral Leukocyte Cultures Without Average Temperature Rise of Culture Medium
R. G. Lattes, and S. Brecher

This study was conducted to examine the chromosomal response to 2450 MHz radiation under circumstances in which gross rises in temperature of the cellular milieu should be detected. Venous blood was obtained from human volunteers, heparinized, and allowed to settle. Leukocytes were transferred to Falcon plastic flasks with culture medium containing serum, phytohemagglutinin and antibiotics. Culture flasks were exposed to microwave radiation for periods of time so that the average temperature of the culture medium did not exceed 38-39° C throughout the total duration of the experiment. Cultures were exposed at culture times which corresponded with G1, S, and G2 phases of the lymphocyte cell cycle during blastogenesis. Dividing cells were accumulated in culture with Colcemid. For light microscopy, cells were fixed, spread on microscope slides and stained; for electron microscopy, cells were lysed at the liquid/air interface of a Langmuir trough, and the surface film was picked up on grids and dried in CO_2 with a critical point apparatus.

Preliminary data indicate that chromosome aberrations in microwave-exposed cells were not induced. No significant features of chromosomal ultrastructure were observed by electron microscopy. Figure 1 shows the electron microscopic appearance of two microwave-irradiated chromosomes.

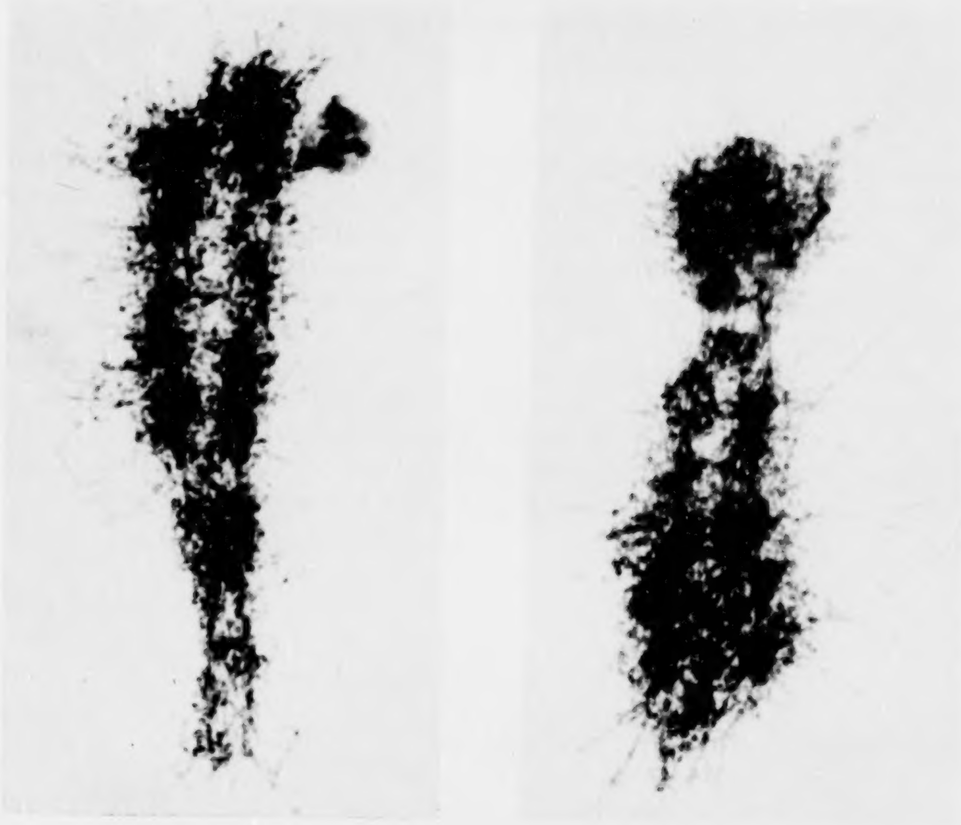

Figure 1. Electron Micrographs of Two Human Metaphase Chromosomes Exposed In Vitro to 2450 MHz Radiation Without an Average Rise in Temperature of Culture Medium.

Disc Polyacrylamide Gel Electrophoresis of Human Immunoglobin G

B. M. Barack, J. Laskey, and J. Horovitz

The immunoelectrophoresis of human immunoglobin G may reveal both strong and faint precipitin lines in samples, whether or not they are irradiated with 2450 MHz microwaves (1). Some of the faint lines appeared to be impurities in the DEAE cellulose chromatographic purification of IgG from Cohn Fraction II. In order to examine the purity of IgG thus prepared, a method was devised for disc polyacrylamide gel electrophoresis of IgG.

Human IgG was isolated from Cohn Fraction II by constant flow fractionation (0.5-1.0 ml/min) at room temperature on DEAE cellulose in 0.0175 M phosphate buffer, pH 6.3, or on Sephadex 6-200 in 0.01 M phosphate buffer, pH 7.5. Eluates comprising the spectrophotometric peak from each column were pooled and concentrated to 4-6 g percent by dialysis in polyethylene glycol.

Discontinuous polyacrylamide gel electrophoresis utilized a Canalco Model 1400 power supply and a 24-gel bath unit. Samples of IgG, 200-400 µg, were added to gel tubes (6.4 cm long, 0.7 cm diameter) which contained 7.5 percent acrylamide. Electrophoresis was for 2 hours at 5 mA/tube in β-alanine-glacial acetic acid buffer, pH 4.5. Following electrophoresis, gels were stained with analine black (1 hour) and destained with 7 percent acetic acid.

The pattern of banding of the eluates was the same, regardless of column separatory matrix. The pattern is shown in Figure 2. The bulk of the sample appears in band 4. With gel staining as a method to estimate protein concentration in each band, the relative concentration of protein per band was 4 > 3 > 1 > 2 > 5.

Combined bands 1 + 2, band 3, band 4, and band 5 were individually isolated from unstained gels, resolubilized in buffer and re-layered on tubes for electrophoresis. With re-electrophoresis, bands 1 + 2, band 3, and band 4 each yielded bands 1 through 4 in a pattern similar to the same four bands as shown in Figure 2. Re-electrophoresis of band 5 yielded only band 5. It must be pointed out, however, that band 5 contained the lowest protein concentration of the five bands.

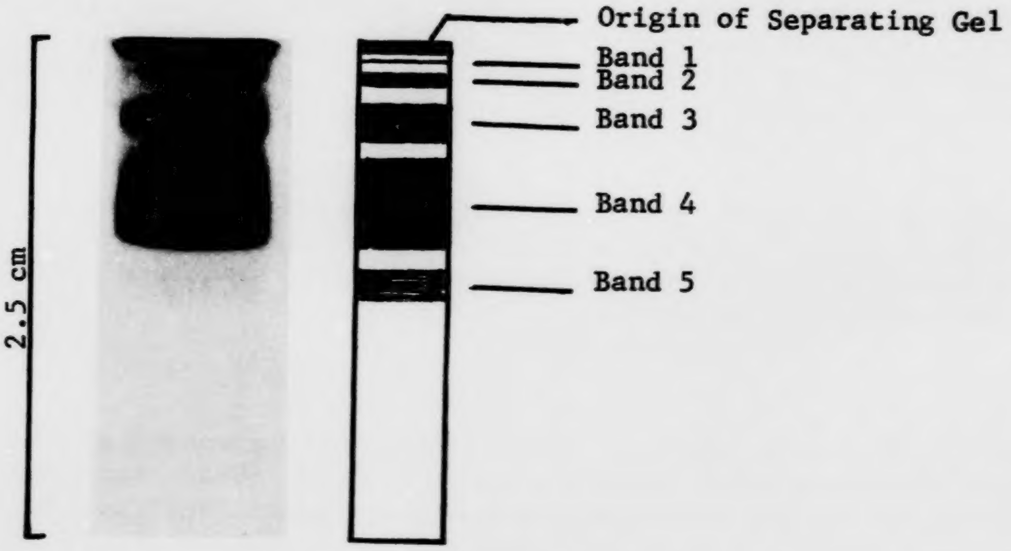

Figure 2. Banding Pattern of Human Immunoglobin G on Polyacrylamide Gel after Purification by DEAE or Sephadex Column Chromatography of Cohn Fraction II.

These data suggest that IgG exists as monomeric and multiple monomeric aggregate forms in aqueous salt solutions.

Reference

1. Kamat, G. P. and D. E. Janes. 1970. Studies on the effects of 2450 MHz microwaves on human immunoglobin G, p. 104-111. In Cleary, S. F., ed. Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings (BRH/DBE 70-2). U.S. Department of Health, Education and Welfare, Public Health Service, Bureau of Radiological Health, Rockville, Maryland 20852.

Chromosomal Analysis of Persons with Body Burdens of Mixed Radioisotopes

E. C. Beyer, B. Shleien, L. Wyche, and L. J. Gillespie

A group of ten individuals with body burdens of mixed radionuclides well below the maximum permissible concentrations permitted for each isotope have been examined by whole-body counting (1) and by cytogenetic techniques. The yield of chromosomal structural changes observed in the individuals was both slightly higher than expected on the basis of radiation exposure records, and apparently unrelated to extent of exposure. In that study, unexposed volunteers served essentially as controls on techniques employed.

In order to expand the size of the sample of exposed individuals and establish a control series of individuals within the work situation, an additional group of individuals with occupational opportunities for exposure to radionuclides was examined cytogenetically. The cytogenetic studies of both groups were performed by peripheral lymphocyte culture techniques.

Analysis of metaphase cells from the cultures is in progress. Although the exposure histories are not known for specific individuals analyzed, the frequency of detection of chromosomal structural changes is less than in the first study.

Reference

1. Athey, T. W. and B. Shleien. 1969. Whole-body counting of some occupationally exposed radiation workers, NERHL 69-3. Northeastern Radiological Health Laboratory, Winchester, Massachusetts.

B. ANIMAL STUDIES

Mortality Patterns of Microwave Irradiated Rat Kangaroo Cells in Culture

K. T. S. Yao, and M. M. Jiles

An earlier report (1) indicated that cultured cells fail to survive power densities of 2450 MHz radiation greater than 1 W/cm^2. To examine some of the characteristics of cell mortality after microwave exposure, a series of experiments was conducted with cells from the ovarian follicle of the rat kangaroo (Potorous tridactylis). Cells in the 30th subculture since establishment of in vitro growth were transplanted into T-30 plastic flasks and permitted to grow for 24 hours. Cells were rinsed with Hanks' salt solution and the flasks were drained before exposing them to microwave radiation for 0-160 minutes at 10, 15, 20, or 50 cm from the antenna. Power densities were estimated at 12.5, 5.6, and 3.1 W/cm^2 at the closer distances; 0.2, 0.3, and 0.5 W/cm^2 were used at 50 cm. After exposure, cells were incubated for 24 hours. The cells have an average cycle duration of 18.6 hours. Trypsin (0.15 percent) was used to detach cells from the flask wall. The cells were concentrated by centrifugation, and stained with trypan blue (1 percent in balanced salt solution). A hemocytometer was used to count 500 cells from each flask. Stained cells were scored as dead. Each treatment was replicated 2-12 times. Average values were calculated, and are presented as percent of control values.

Ten exposure series were performed with three flasks standing side by side. Survival appeared different in the center flasks than in the flanking flasks, with fewer cells surviving in the center flasks. In the remainder of experiments culture flasks were exposed singly in order to reduce the apparent "position effect."

Figure 1 shows the surviving fraction of exposed cells as a function of duration of exposure to three nominally high power densities. There appears to be an exposure duration with minimal effect, < 2 minutes at 12.5 W/cm^2, < 6 minutes at 5.6 W/cm^2, and < 20 minutes at 3.1 W/cm^2. Relative survival decreases as the power density increases. Survival curves are similar at 3.1 W/cm^2 and 5.6 W/cm^2, with the slopes of the linear portions of the survival curves, as plotted on a log-probit scale, parallel to each other. The survival curve after 12.5 W/cm^2 exposure is different from the other curves.

In order to examine the increase of temperature in the culture flasks, they were filled with culture medium 199. The flasks were exposed to microwave radiation at a distance of 10 or 15 cm from the antenna. Irradiation was interrupted at intervals of 10 seconds to determine the temperature of the medium with a mercury thermometer. Temperature measurement required about 30 seconds. Figure 2 shows the temperature rise of the medium. It is clear that temperatures at which cells cannot survive were rapidly reached in the medium. The volume difference between cells in culture flasks and the medium in flasks indicates that the heating of the two types of preparations was not equivalent.

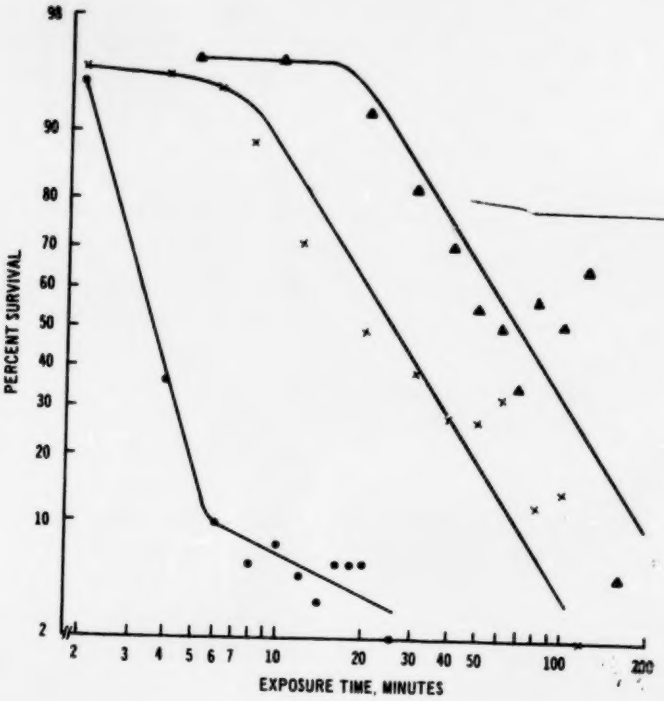

Figure 1. Log Probit Plot of Survival of Rat Kangaroo Ovarian Follicular Cells Exposed to 2450 MHz Radiation. Symbols: ● = 12.5 W/cm^2; x = 5.6 W/cm^2; ▲ = 3.1 W/cm^2 of estimated power densities.

Survival and growth of cells in cultures exposed at 50 cm from the antenna (0.2 - 0.5 W/cm^2) have been variable in 6-8 replications at each power density. It is not possible, therefore, to reach any conclusions about the experiments at the 50 cm distance.

Reference

1. Yao, K. T. S., and M. M. Jiles, 1970. Effects of 2450 MHz Microwave Radiation on Cultivated Rat Kangaroo Cells, p. 123-133. In Cleary, S. F., ed., Biological Effects and Health Implications of Microwave Radiation, Symposium Proceedings (BRH/DBE 70-2). U.S. Department of Health, Education, and Welfare, Public Health Service, Bureau of Radiological Health, Rockville, Maryland 20852.

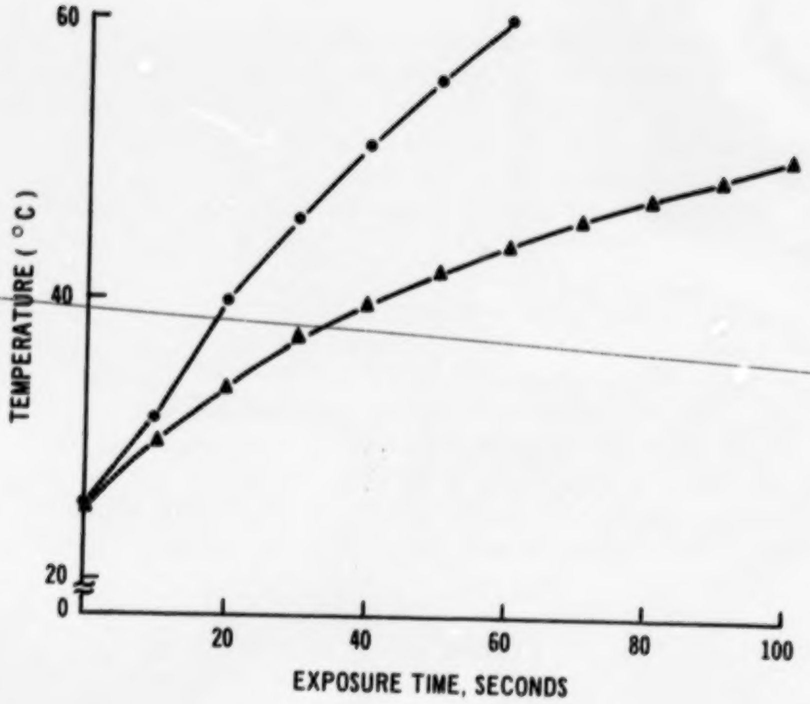

Figure 2. Temperature Rise in Culture Medium Exposed to Microwaves During 10-Second Intervals Which Were Separated by Approximately 30 Seconds to Measure Temperature. Medium was irradiated at 10 cm (●) or 15 cm (▲) from the antenna.

Haploid Cells in Rat Kangaroo Corneal Endothelium Cultures and X-Ray Induced Chromosome Aberrations

K. T. S. Yao

Cell cultures were initiated in February 1967, from the corneal endothelium from a female rat kangaroo (*Potorous tridactylis*). For the first 20 passages cells were cultured in Eagle's minimum essential medium supplemented with 20 percent calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate. One hundred units of penicillin and 100 μg equivalents of streptomycin were added per ml of complete medium. After the 20th passage medium 199 was substituted for the Eagle's medium. For the first six passages the cells were flat and epithelial-like, and formed monolayers. About the 16th passage virtually all of the cells in the cultures were fibroblast-like, and grew in multiple layers.

The culture morphology has persisted to the present time. The chromosomal constitution was determined after the 20th passage. A majority of mitotic cells were diploid, and a few mitotic cells were polyploid. Between 0-20 percent of mitotic cells contained the haploid chromosome complement, five autosomes and one x chromosome. The incidence of these "haploid" cells has been as high as 32 percent in subsequent passages.

To examine radiation-associated effects, these cultures were rinsed with Hanks' balanced salt solution and exposed to 0, (control) 20, 50, 100, and 150 R of x rays (250 kVcp; HVL of 0.55 mm Cu; 15 mA; target to specimen distance, 120 cm; exposure rate, approximately 31 R/min). After irradiation fresh medium was added to the cultures before incubating them at 37° C. Cells were fixed at 6, 12, 24, 36, and 48 hours after irradiation. Mitotic cells were accumulated with Colcemid (0.03 μg/ml of medium) for 3 hours prior to fixation.

The frequency of haploid cells in cultures increased with the age of the culture. Between 0-100 R of x rays no effect of irradiation was observed on the frequency of haploid cells in the cultures. The number of haploid cells per culture was inversely related to the mitotic index of the culture (correlation coefficient of -0.70). The relationship between haploid cell numbers, mitotic index of culture, and x-ray exposure, is shown in Table 1.

Table 2 shows the chromosomal aberrations observed in diploid and haploid cells in cultures. The average total number of breaks per cell was 0.2950 for diploid cells, and 0.0863 for haploid cells. The number of breaks per cell per R of x rays ranged between 0.0025 and 0.0142 for diploid cells and between 0.0009 and 0.0046 for haploid cells.

The diploid cells registered approximately three times more chromosomal damage (152/950) than the haploid cells (52/950). The calculated number of breaks per cell per R was highest at 20 R in both haploid and diploid cells. The significance of this observation, if any, is not known.

TABLE 1. AVERAGE NUMBER OF HAPLOID CELLS PER 50 DIPLOIDS AND AVERAGE MITOTIC INDEXES OF THE ENDOTHELIUM CELL CULTURE

Hours After Irradiation	0 R		20 R		50 R		100 R		150 R		Average	
	No. Hap.	Mit. Ind. (%)	No. Hap.	Mit. Ind. (%)	No. Hap.	Mit. Ind. (%)	No. Hap.	Mit. Ind. (%)	No. Hap.	Mit. Ind. (%)	No. Hap.	Mit. Ind. (%)
6	13.8	8.4	11.5	12.9 $t=4.8^a$	12.0 $t=5.8^a$	12.1 $t=5.8^a$	--	--	--	--	12.4	11.1
12	--	--	12.8	6.2	11.3	5.3	11.5	5.6	--	--	11.9	5.7
24	12.0	4.2	15.0	4.3 $t=0.1^b$	16.5	4.5 $t=0.1^b$	17.0	4.3 $t=0.1^b$	14.5	3.8 $t=0.1^b$	15.0	4.2
36	--	--	14.8	1.4	14.8	0.7	14.3	1.1	--	--	14.6	1.1
48	20.8	0.6	20.0	0.3 $t=2.5^c$	22.8	0.1 $t=4.5^a$	24.3	0.1 $t=4.5^a$	22.0	0.2 $t=3.4^a$	22.0	0.3
Average	15.5	4.4	14.8	5.0	15.6	4.5	16.8	2.8	18.3	2.0		

a. Difference from control significant at a probability of 0.01.
b. Difference from control not significant.
c. Difference from control significant at a probability of 0.05.

TABLE 2. NUMBER OF CHROMOSOME ABERRATIONS IN X-IRRADIATED CELL CULTURES

Exposure R	No. Cells Observed	Ch'tid Br.	Iso-ch'tid Br.	Dicen-trics (2)	Rings (2)	2nd Const. (0)	Total No. Br.[a]	No. Br. Per Cell	No. Br. Per Cell Per R
					Diploid Cells				
0	150	0	0	1	0	3	2	0.0133	--
20	250	3	2	30	3	2	71	0.2840	0.0142
50	250	1	5	18	8	5	58	0.2320	0.0046
100	200	9	9	25	1	0	70	0.3500	0.0035
150	100	4	5	14	0	0	37	0.3700	0.0025
Total	950	17	21	88	12	10	238	0.2950	--
					Haploid Cells				
0	150	0	0	0	0	0	0	--	--
20	250	1	0	9	2	5	23	0.0920	0.0046
50	250	1	0	4	2	1	13	0.0440	0.0009
100	200	2	3	5	2	3	19	0.0950	0.0009
150	100	0	2	5	1	0	14	0.1400	0.0009
Total	950	4	5	23	7	9	69	0.0863	--

a. Each dicentric, ring, or chromatid exchange was counted as 2 breaks as indicated in parentheses. Secondary constrictions were not counted as breaks.

Chromosomal Analysis of Rat Mammary Tumor Cells in Culture

S. A. Knadle, and L. J. Gillespie

In a collaborative study of cell cultures originally derived from a papillary cystadenoma of the mammary gland of the Osborne-Mendel rat, chromosomal analysis has been performed. Karyotypes of cells with consistent patterns of chromosomal changes are shown on p. 211. The significance of the chromosomal changes is unknown with respect to the ability of the cells to induce tumors in animals.

Effects of ^{89}Sr on the Incidence of Chromosomal Aberrations in Leukocytes of Adolescent Cats

S. A. Knadle, S. Brecher, E. Berman, and W. L. Lappenbusch

As part of their study of the delayed effects of ^{89}Sr on cats, the Toxicologic Studies Section wanted to examine the feasibility of monitoring the radiation-related chromosomal damage. Strontium 89 has a physical half life of 50.5 days and emits a negatron with 1.463 MeV maximum energy and a gamma ray with 0.91 MeV energy. In the pilot study the peripheral blood was selected because blood is easy to obtain, leukocytes grow well in short-term cultures and sampling is of low risk to the test animals.

Twelve adolescent cats were force-fed a capsule containing 0, 25, 50, or 100 µCi ^{89}Sr daily for 30 days. Approximately 30 days after the last feeding, 5 ml of blood was taken from the anterior jugular vein, heparinized, and allowed to settle. Leukocytes at a concentration of 2.5 x 10^6 cells/ml were added to NCTC-109 supplemented with 30 percent serum, 1 percent glutamine, 1 percent penicillin-streptomycin mixture, and 0.01 percent pokeweed mitogen. Cultures were maintained at 39° C for 48 hours. Mitotic cells were collected with Colcemid during the final 3 hours of culture.

The chromosomal data obtained from the cultures are shown in Table 3. Percent aberrant cells ranged between 0 and 3.6 before exposure, and between 1 and 8 at 30 days after last exposure. The magnitude of the trend toward increased chromosomal damage in exposed cats would require about 275 cats for an adequate test of possible statistical significance of the difference.

TABLE 3. EFFECT OF 30 DAILY ADMINISTRATIONS OF ^{89}Sr ON THE PERCENTAGE OF INDUCED CHROMOSOMAL ABERRATIONS. TOTAL SAMPLE SIZE WAS 12 CATS

μCi ^{89}Sr Administered Daily for One Month	Animal Number	Cells With Chromosomal Aberrations, %		Average Difference (%)
		Immediately Before First Exposure	Thirty Days After Last Exposure	
0	1457	2	2	0.2
	1487	0	3	
	1501	3.6 (55)[a]	2	
25	1455	1	4	1.0
	1489	0	1	
	1500	2	-[b]	
50	1456	0	2	1.8
	1486	1	1.4 (71)[a]	
	1502	0	5.6 (125)[a]	
100	1460	1	2	1.3
	1488	2	-[b]	
	1491	1	8	

a. Number of cells counted other than 100.
b. Cells did not grow in culture.

In Utero Exposure of Cats to ^{89}Sr and Chromosomal Damage in Leukocytes

S. Brecher, S. A. Knadle, W. L. Lappenbusch, and E. Berman

The utility of using the peripheral leukocyte to estimate chromosomal damage in cats that had been exposed in utero to ^{89}Sr was investigated in a pilot study. Blood was obtained from dams and surviving progeny of the gestational period during radiostrontium treatment. Progeny were adolescent when blood was obtained. Leukocytes from the blood were incubated in culture medium for 72 hours at 37-38° C. Exposure histories of the dams and percent of cells with aberrant chromosomes are shown in Table 4. In the three progeny of the untreated cat, 0-4 percent of mitotic cells had aberrant chromosomes. In the progeny of treated cats, 0-7 percent of mitotic cells had aberrant chromosomes. Figure 3 shows a typical chromosomal spread from the cat leukocyte cultures.

TABLE 4. INCIDENCE OF CELLS WITH ABERRANT CHROMOSOMES FROM CATS EXPOSED TO RADIOSTRONTIUM IN UTERO AND POSTNATALLY VIA DAM'S MILK

Radiation History of Dam	Percent of Aberrant Cells in Dam	Sex of Progeny	Percent of Aberrant Cells in Progeny
No exposure	No analysis	♀	0
		♀	4
		♀	2
62.5 μCi ^{89}Sr/day orally for 65 days of gestation; unknown dose ^{123}I intravenously postpartum; completed nursing; 93.75 μCi ^{85}/^{89}Sr/day orally for 60 days	12	♀	0
		♀	4
		♀	2
		Litter nursed for 52 days	
125 μCi ^{89}Sr/day orally for 63 days of gestation	No analysis	♂	5
		♂	4
		Litter nursed for 49 days	
62.5 μCi ^{85}/^{89}Sr/day orally for 65 days of gestation	No analysis	♂	7
		♀	0
		Litter nursed for 64 days	

NOT REPRODUCIBLE

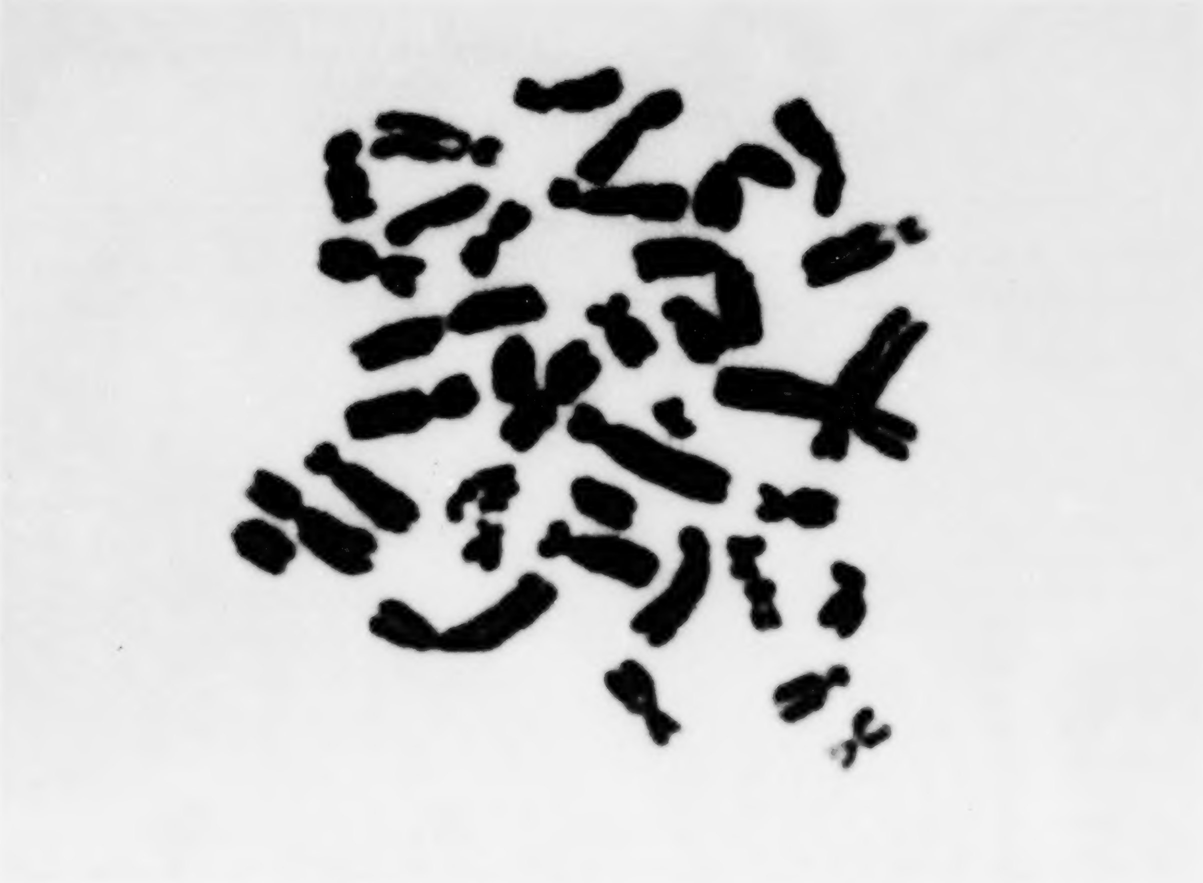

Figure 3. Colcemid-Metaphase from a Peripheral Blood Leukocyte Culture of the Domestic Cat.

Cell Cultures From Thyroids of Adult Chinese Hamsters

L. J. Gillespie, and W. M. Leach

In the Radiation Bio-Effects Summary Report, 1969, we reported the occurrence of an elongated No. 5 chromosome in cultures of Chinese hamster thyroid cells between the second and fourth serial passages in vitro, and the concomitant aneuploidy of the cells. We also reported the vesiculation and granulation of the cells at about the 30th passage. We report here some additional observations on these cells.

Formation of Cell Hybrids

Cells derived from the Chinese hamster thyroid and WI-38 cells were mixed in equal proportions. The mixed cell culture was maintained and observed through 25 serial passages. Marked polyploidy of cells was observed during the first 16 passages, and accounted for about 50-75 percent of cells in division. Figure 4 shows cellular polyploidy, as well as a high degree of granulation. Also present were chromosomal breakage and rearrangements (Figure 5). From the 16th passage to the 20th passage, the predominant chromosomal constitution of the cells was the normal Chinese hamster karyotype. The elongated No. 5 chromosome and the small extra metacentric chromosome(s) were not observed in any significant proportion of mitotic cells. Figure 6 shows two adjacent metaphase spreads of chromosomes in the cells during the passages 16-20. Beginning in passage 20, however, the elongated No. 5 chromosome began to reappear in dividing cells. It became evident even in polyploid cells by passage 25, the last period of observation of the cells. In the residual polyploid cells an "erosion"--loss of stainability--of chromosomes in the complement was noted (Figure 7).

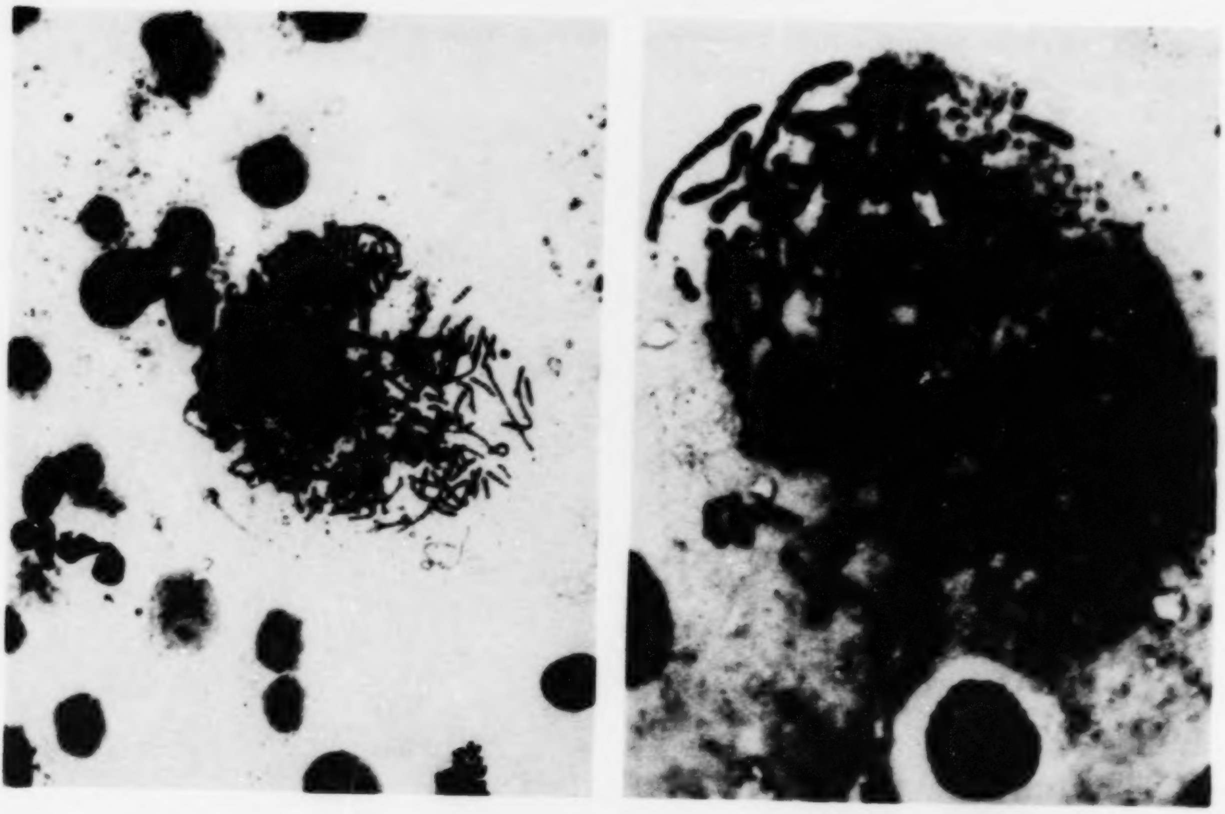

Figure 4. Polyploidy and Granulation in Dividing Cells from Mixed Cultures of Chinese Hamster Thyroid and Human WI-38 Cells.

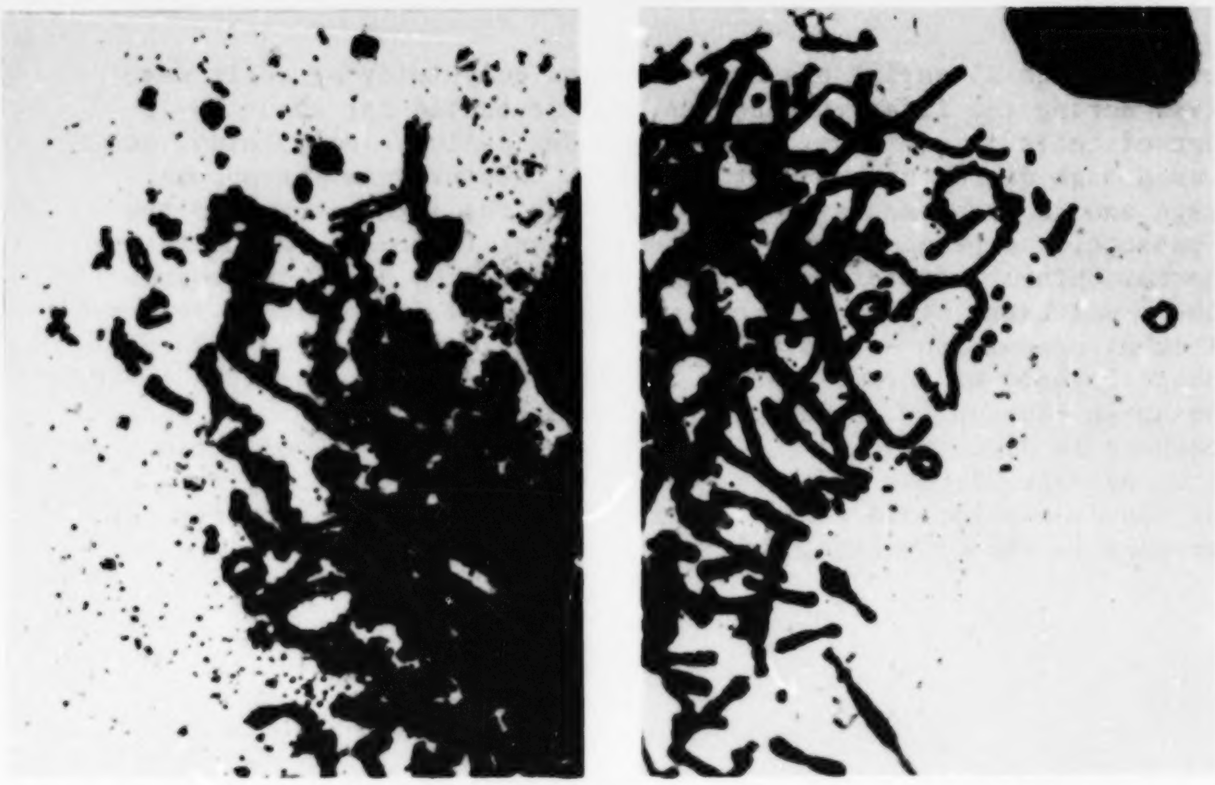

Figure 5. Aberrant Chromosomes in Polyploid Cells from Mixed Cultures of Chinese Hamster Thyroid and Human WI-38 Cell Lines. Polyploidy and chromosomal rearrangements are observed during about 16 culture passages after mixing the cell types.

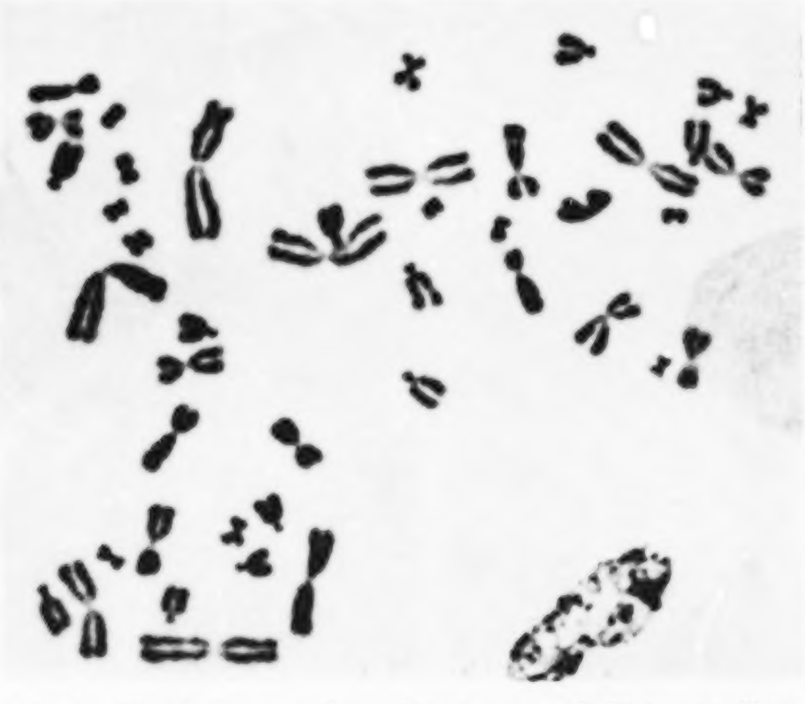

Figure 6. Metaphase Chromosomes from Mixtures of Chinese Hamster Thyroid and WI-38 Cell Lines. The morphologically normal hamster chromosome complement predominates during passages 16-20 after mixing the cells.

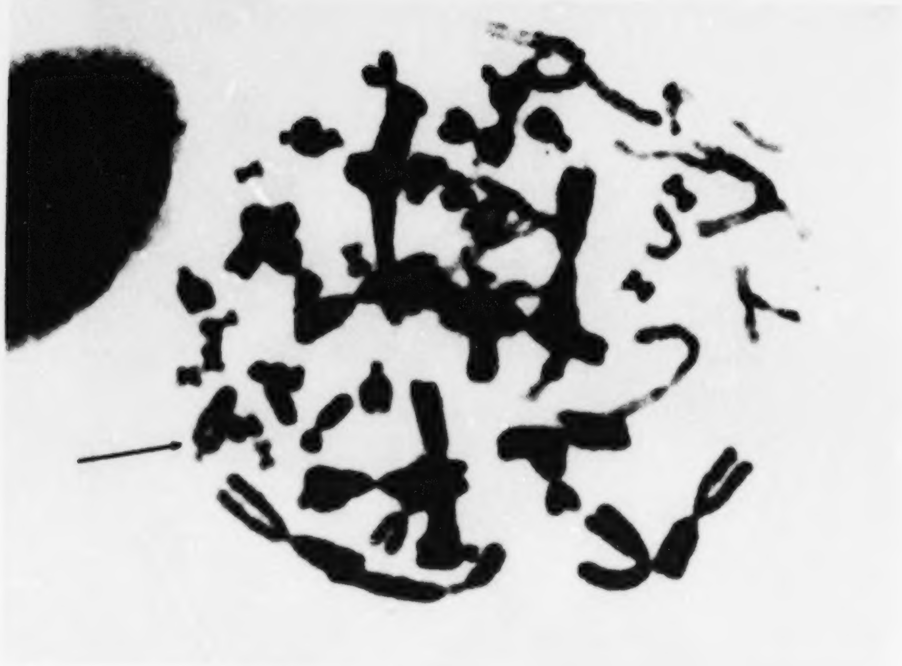

Figure 7. Polyploid Cell at the 25th Culture Passage after Mixing Chinese Hamster Thyroid and Human WI-38 Cells. The "marker" chromosome is present (arrow). Numerous chromosomes appear eroded.

Medium used to support growth of the Chinese hamster cells was filtered with a Selas filter (4.5μ pore size) and was added to monolayers of WI-38 cells in T-75 Falcon flasks. After two hours the medium was removed and replaced with fresh medium. After 24 hours the cells were seeded in Leighton tubes. Growth of the treated cultures was increased in comparison with control cultures, as judged by the frequency of mitotic figures. Approximately 2 percent of mitotic figures in the treated cultures showed endoreduplication of chromosomes (Figure 8).

Granules

Granulation has been observed in the cultured cells from the Chinese hamster thyroid, and in heterospecific cell hybrids of thyroid/WI-38 cells. The granules vary in size to some extent, as if they coalesce or aggregate. In non-dividing cells, they tend to be primarily localized in the cytoplasm, although nuclear stainability may interfere with their visualization. In dividing cells, the

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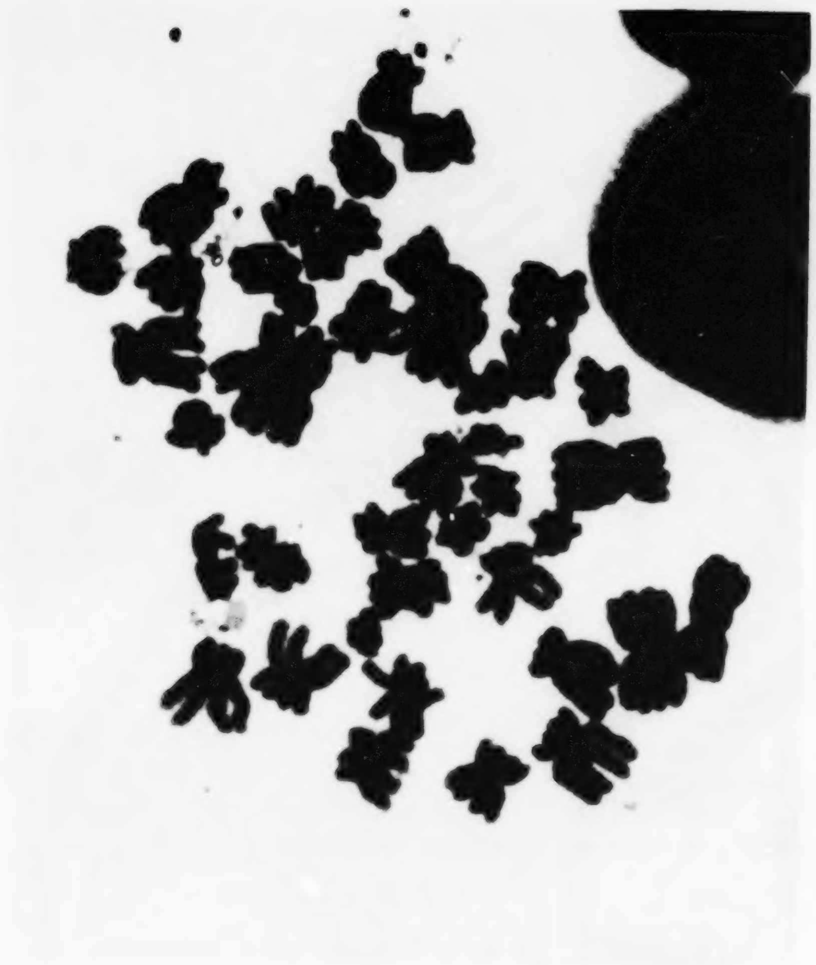

Figure 8. Endoreduplication of Chromosomes of WI-38 Cells Treated with Medium from the Chromosomally Marked Chinese Hamster Cell Line.

granules appear associated with chromosomes. Figure 9 shows a non-dividing cell with cytoplasmic granules. Cells of the WI-38 line show granulation after treatment with medium from the thyroid cells described above. Figure 10 shows untreated and treated metaphase WI-38 cells. Chinese hamsters which have been injected with the cultured thyroid cells have similar sorts of granules in blood cells, primarily the granulocytic white cells. The extent to which similar-appearing granules may appear normally in the hamster breeding colony is not known at the present time.

Tumorigenicity

J. N. Shively, A. McTighe

Cells with the elongated No. 5 chromosome have been injected into Chinese hamsters in three regions: intraperitoneal, intraocular, and

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Figure 9. Granules in a WI-38 Cell Treated with Medium from the Chromosomally Marked Chinese Hamster Cell Line.

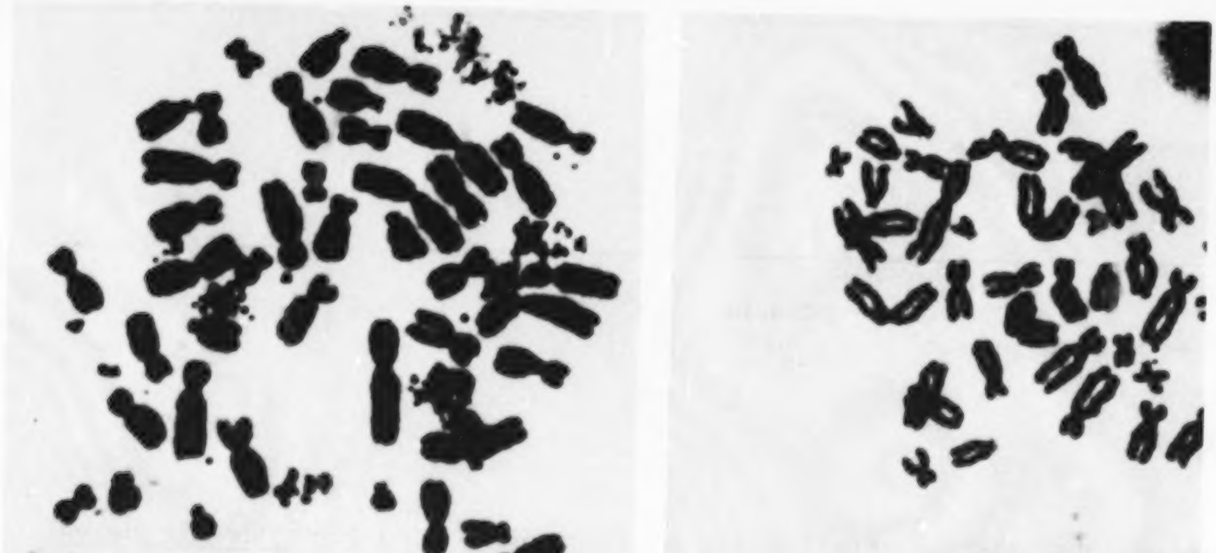

Figure 10. Metaphase WI-38 Cells. Granules cluster near chromosomes in a cell from a culture treated with medium from the chromosomally marked Chinese hamster cell line (A). (B) shows a cell from an untreated culture.

intracranial. First attempts were intraperitoneal. Encapsulated nodules were observed in injected animals. The nodules were usually free on mesenteries and were particularly near the pancreas. Nodules were also observed in the pleural cavity. About 90 percent of injected animals exhibited intracoelomic nodules, which disappeared about 14 days after injection of the cells.

Some nodules were removed aseptically and crushed in tissue culture flasks. After 2-3 weeks of culture, the elongated No. 5 chromosome was apparent in half of the mitotic cells. The growth of the cells in culture was also characteristic of the uninjected thyroid cell cultures, i.e., clumping of cells.

Table 5 shows the schedule of injection, and results of intra-animal growth of cells. Tumors observed to date have been fibrosarcoma in type, and appear to invade surrounding tissues. Single cell isolates of tumors have been reinjected into healthy animals and about 90 percent of the animals developed tumors.

TABLE 5. TUMORS IN CHINESE HAMSTERS AT VARIOUS INJECTION SITES

	Animal Age at Injection		
Site of Injection	<24 hour old	10-20 days old	>30 days old
Intraperitoneal		±	±
Intraocular			++
Intracranial	++	++	

± growth of nodules present
++ tumors present

Developmental and Genetic Testing of Drosophila With 2450 MHz Microwave Radiation

E. C. Beyer, T. L. Pay, and E. T. Irwin, Jr.

The question of potential mutagenicity of microwave radiation has been explored with *Drosophila melanogaster* as a genetic test system. Male flies were removed from Oregon-R stocks within 24 hours after ecdysis. Five males were placed in an acrylic capsule 18 cm from the antenna and exposed to 2450 MHz radiation generated at forward power of 2.1, 2.75, and 3.0 kW. The 2.75 kW level was lethal to two of five flies. Each irradiated fly was serially placed with two virgin Muller-5 females at 24-hour intervals for a total of 15 days (30 females per male). Sham exposed control flies at each radiation level were similarly mated. The number of days to eclosion of F_1 progeny was used as generation time. The F_1 broods were counted and sexed on the 17th day of culture. From each daily brood approximately 45 F_1 pairs from exposed flies and 15 F_1 pairs from control flies were selected from the 3.0 and 2.75 kW series for F_2 analysis of sex-linked recessive lethal mutations by the Muller-5 method.

The F_1 generation times showed no effect. Total F_1 production and sex ratios (female/total) showed statistically significant daily deviations, but no trends were present. Analysis of F_2 progeny were performed for a total of 749 irradiated X chromosomes in P_1 males and 147 X chromosomes in control males. Sex-linked lethal mutations were not observed. Table 6 shows the results of the present study in comparison with results reported from another laboratory.

TABLE 6. F_2 STUDY FOR SEX-LINKED LETHAL MUTATIONS UTILIZING THE MULLER-5 CHROMOSOME

	No. F_2 Broods	No. Lethals Expected[a]	No. Lethals Observed
	3.00 kW		
Exposed	373	1.12	0.0
Control	78	0.02	0.0
	2.75 kW		
Exposed	376	1.13	0.0
Control	69	0.014	0.0

[a] Expected frequencies taken from data published by Mickey (1) at 30-60 MHz.

The present results do not show a large rise in sex-linked recessive mutations after 2450 MHz continuous wave radiation. In the earlier study (1), a ten-fold rise was observed after pulsed radiation in the frequency range 30-60 MHz, generated by capacitor plates. The present results may also be in contrast to the correlation between paternal exposure to radar and Down's syndrome in offspring (2), and to reports of chromosomal aberrations after microwave radiation (1, 3). It should be pointed out that the extent of coupling between Drosophila and the 2450 MHz microwave field is in question because of the small size of the fly. Clustering of fles could account for the observed lethality. We have shown that the number of flies exposed to microwaves affects the observed lethality.

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APPENDIX A

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APPENDIX B

PAPERS PREPARED FOR PRESENTATION
JANUARY - DECEMBER 1970

Angleton, G.M., R.J. Garner, A.C. Lee, R.D. Phemister, and R.W. Thomassen. Age variations in radiosensitivity in the developing beagle. 2. Post-natal radiosensitivity. IVth International Congress of Radiation Research, Evian, France, June 29 - July 4, 1970.

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Beyer, E.C., and T.L. Pay. Microwave effects on genetic transmission. Fifth Annual Microwave Power Symposium, Scheveningen, Holland (The Hague), October 7-9, 1970.

Brecher, S. X-ray effects on the nucleolus and on its role in chromosome distribution. Fifth Annual Joint Meeting of the Clinical Society and the Commissioned Officers Association, Washington, D.C., March 31 - April 3, 1970.

Bretthauer, E.W., A.L. Mullen, and A.A. Moghissi. Milk transfer comparisons of different chemical forms of iodine. American Chemical Society, Chicago, Illinois, September 13-18, 1970.

Budd, R., R. Chiacchierini, D.G. Hazzard, J. Rolofson, and S.J. Smith. Comparative response to 30 and 250 kVp x-rays of the mouse hematological system. Radiation Research Society, Dallas, Texas, March 1-5, 1970.

Carpenter, R.L. Biological effects of non-ionizing radiation. American Public Health Association, Houston, Texas, October 26-30, 1970.

Fred, R.K. Kinetics of phagocytosis in the liver. Sixth International Congress of the Reticuloendothelial Society, Freiburg (B.R.), July 29 - August 1, 1970.

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Harris, J.Y. National inventory of electronic products. Fourth Annual Health Physics Society Midyear Symposium, "Electronic Product Radiation and the Health Physicist", Louisville, Kentucky, January 28-30 1970.

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Yao, K.T.S. Radiosensitivity of haploid cells in rat kangaroo corneal endothelium cultures. Tissue Culture Association, Washington, D.C., June 15-18, 1970.

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RADIATION BIO-EFFECTS SUMMARY REPORT, JANUARY - DECEMBER 1970.

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ABSTRACT: This report summarizes the activities of the Division of Biological Effects during 1970. The investigations of Epidemiologic Studies Branch and the Experimental Studies Branch which comprise the Division are discussed. Titles of papers that division investigators have prepared for presentation and those appearing in print during the year are listed.

KEYWORDS: Animal; Human; Long-Term Effects; Microwave; Nonionizing Radiation; Radiation Research; Radiobiology; Radionuclide; X Ray.

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